SEM morphological studies of carbonates and the search for ancient life on Mars

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1. Introduction

Next space missions will investigate the possibility of extinct or extant life on Mars. 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 (Orofino et al. 2007, 2009). 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 to 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 that can be considered as good analogues for putative Martian life forms. Considering that the microstructures of biogenic carbonate are different from those of abiogenic origin (D'Elia et al., 2006), we investigated the micromorphology of shells, skeletal grains and microbialites at different scale with a Scanning Electron Microscope. The results show that this line of research may provide an alternative and complementary approach to other techniques developed in the past by our group to distinguish biotic from abiotic carbonates. Below we present some results that can be of valuable interest since they demonstrate the utility for a database of images concerning the structures and textures of relevant carbonate minerals. Such data may be useful for the analysis of Martian samples, coming from sample return missions or investigated by future in situ explorations, aimed to characterize the near-sub surface of Mars in search for past or present life.

2. Sample description and preparation

The samples analysed in this work are listed in Table 1. The first two are fossil shells composed of aragonite, a metastable phase of calcium carbonate ($CaCO_3$), while the others are skeletal fossils of organisms (coral, sponge and algae) and microbialites. All samples are made of calcium carbonate, calcite or aragonite, with some silicates in the case of the microbialites. A calcite mineral rock of abiotic origin is also included for comparison. The estimated geological ages of the samples are also reported in Table 1 where 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 fossil shells of two marine gastropod molluscs, already studied by Orofino et al. (2010), have been collected in clay deposits located at two different sites, about 30 km apart, in the Salento Peninsula, Italy. The Xenophora gastropod comes from a Pleistocene deposit and in particular, in relation to stratigrapy, from a whitish calcarenite with an upper greenish clayey-sandy interval, having an elevated paleontological interest for the abundance of the fossil associations with excellently preserved taxa.

The Ampullinopsis crassatina, that biostratigraphical indications allowed to assign to the late Oligocene, was collected from a lignite clayey deposit, overlapped on a blanket of mineralized residual deposits (bauxitic residual deposits) resting on the local carbonatic basement.

The samples S/L, S1A and U2 are skeletal organisms (coral, sponge and algae respectively) embedded in microbial carbonates such as sample S/L(M). They have been selected within three rock specimens that developed, in time, in two distinct palaeoecological conditions, characteristic of Alpe di Specie and Punta Grohmann carbonate outcrops, in the Dolomites, Italy. The composition of S/L and S1A samples is dominated by the micritic fraction, 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, are sometimes organized in stromatolitic laminae or thrombolitic fabric. Instead, the U2 sample is characterized by subcentimeter skeletons intimately associated

Table 1
Studied samples listed in order of confidence of biological origin. In the last column, we report the types of micromorphology of each sample (DP: defined pattern; MM: massive micromorphology).

Sample	Description	Composition	Geologic period/epoch	Micromorphology
Xenophora	Gastropod	Aragonite	Pleistocene (1.8-0.1 Ma)	DP
Ampullinopsis crassatina	Gastropod	Aragonite	Oligocene (34-23 Ma)	DP
S/L	Coral	Aragonite, Calcite	Upper Triassic, Carnian (229-217 Ma)	DP/MM
S1A	Sponge	Calcite, Aragonite	Upper Triassic, Carnian (229-217 Ma)	DP
U2	Alga	Calcite	Middle Triassic, Ladinian (237-229 Ma)	DP/MM
S/L(M)	Microbialite	Calcite, silicates	Upper Triassic, Carnian (229-217 Ma)	DP/MM
GE	Stromatolite	Calcite, silicates (traces)	Upper Jurassic, Tithonian (151-146 Ma)	DP/MM
Calcite	Abiotic mineral	Calcite	—	MM

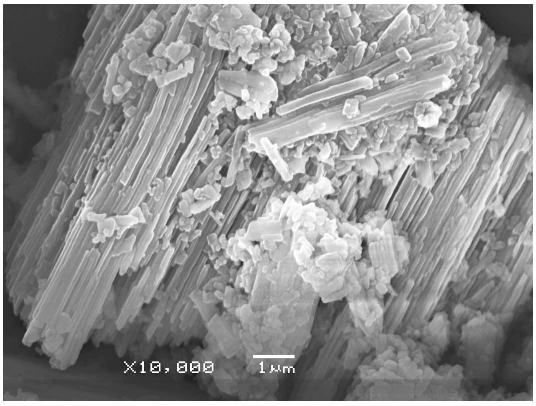

Figure 1. SEM image of the *Xenophora* particles.

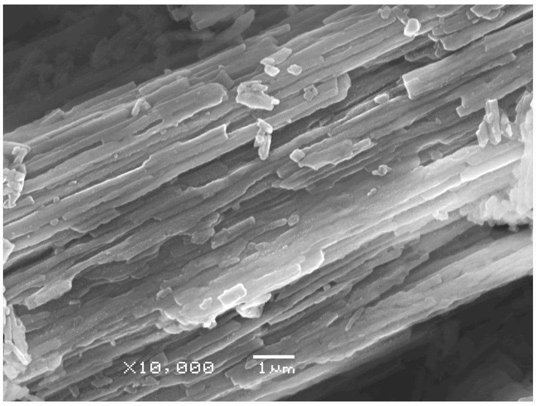

Figure 2. SEM image of the *Ampullinopsis crassatina* particles.

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.

The sample GE, was collected in an area in which stromatolites developed in stressed environments with alternate deposition of oolitic limestone and evaporites (Jahnke and Ritzkowski, 1980).

In order to obtain fine particulate materials for SEM morphological analyses, the fossil shells (*Xenophora* and *Ampullinopsis crassatina*) and the mineral calcite were ground with a mechanical mortar grinder and then the size fraction between 20 μm and 50 μm was sieve selected for our investigation. The other specimens, instead, were collected from specific areas of the rock samples using a thin drill bit.

In the end, the various samples were placed inside the SEM chamber in high vacuum mode and morphologically analyzed with the secondary electron detector. 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 SEM JEOL JSM 6480LV, equipped with an iXRF Systems EDS Sirius SD spectrometer for the elemental composition.

In Figs. 1 and 2 typical SEM images of parti-

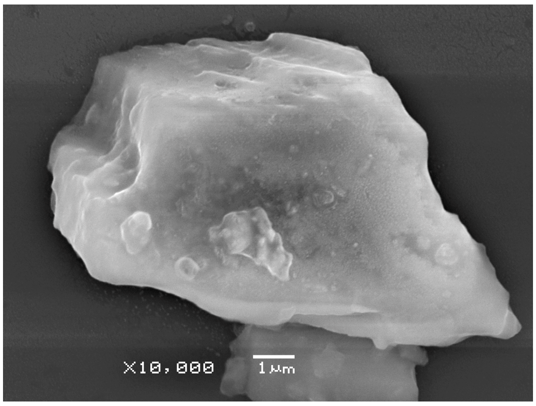

Figure 3. SEM image of abiotic calcite mineral particles.

cles of the two fossil shells are reported while in Fig. 3 the micrograph of mineral calcite grains is shown. It is evident the well-defined crystal pattern of the shells particles compared with the massive micromorphology of the mineral crystals imaged at the same scale.

Similar morphologies at micrometric scale are observable in Fig. 4a and from Fig. 5 to Fig. 8 for the skeletal organisms (samples S/L, S1A and U2), microbialites and stromatolites (samples S/L(M) and GE respectively). Except for the case of S1A (sponge), in which all the particles have the same crystalline structure (see Fig. 5 and last column of Table 1), in the other samples some grains exhibit a well-defined pattern (labelled DP in Table 1) while others show a massive micromorphology (labelled MM) as the abiogenic calcite crystals. As an example, we can refer to the difference between panel a and b in Fig. 4 (S/L sample) and the comparison between Fig. 4b and Fig. 3 (calcite) that indicate an analogous arrangement.

Furthermore, we note that within those biogenic samples showing a well-defined pattern the biogenic samples can be divided in two categories: those having a "laminar" pattern (coral and sponge, Fig. 4a and Fig. 5) similar to those of the fossil shells and those having a granular pattern of small crystals (algae, microbialites and stromatolites, Figs. 6, 7 and 8). Even if such differences could be ascribed to a diverse degree of complexity of the involved forms of life, we tentatively speculate that they can be linked to the diverse type of biomineralization. In fact, microorganisms are remarkably adept at forming mineral phase and this process can occur in two different way. The first involves mineral precipitation in the open environment, without any apparent control by the cell over the mineral product. This process was defined by Lowenstam (1981) as "biologically induced biomineralization", with mineral forming only as a by-product of the cells metabolic activity or through

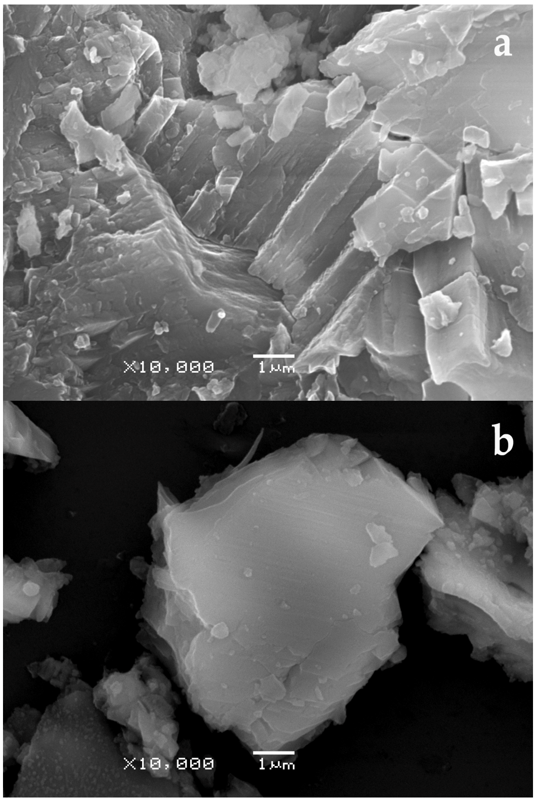

Figure 4. SEM images of the coral particles (samples S/L). Some particles show a well-defined pattern (panel a) while others a massive micromorphology (panel b).

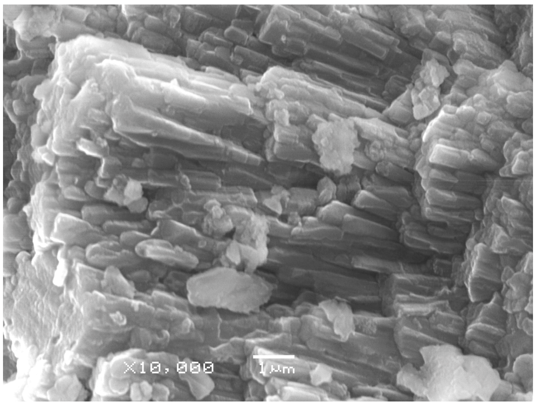

Figure 5. SEM image of the sponge particles (sample S1A).

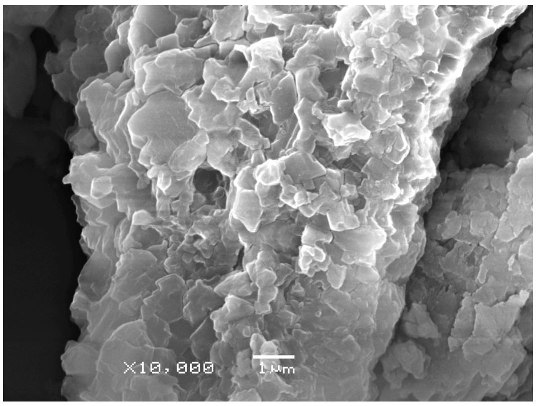

Figure 6. SEM image of the algae particles (sample U2).

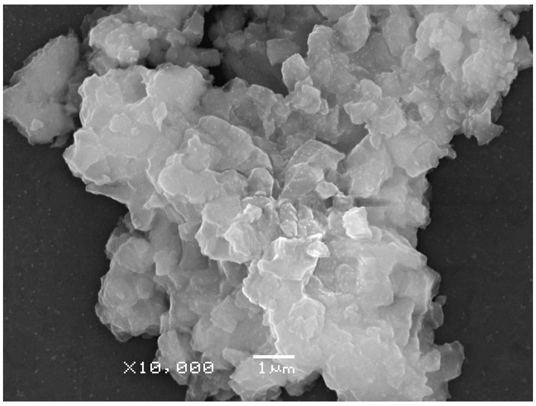

Figure 7. SEM image of the microbialite particles (sample S/L(M)).

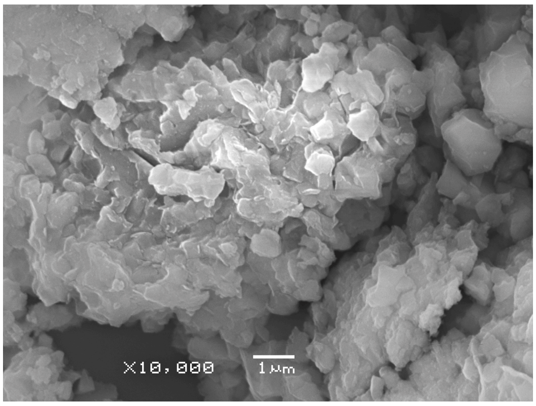

Figure 8. SEM image of the stromatolites particles (sample GE).

its interaction with the surrounding aqueous environment (Konhauser, 2007). By contrast, the second way, "biologically controlled biomineralization", is completely regulated, allowing the organisms to precipitate mineral that serve some physiological purpose. This process is specifically designed to form minerals through the development of intracellular (within the cytoplasm) or epicellular (on the cell wall) organic matrices, into which specific ions are actively introduced and their concentrations controlled in order to achieve the right mineral saturation states. Since the mineralization site is isolated from outside the cell by a barrier through which ions cannot freely diffuse, minerals can form anyway, in spite of thermodynamically unfavourable external conditions (Konhauser, 2007).

To investigate the distribution of these different crystal patterns of particles, we have morphologically examined more than 50 grains of each sample and we have summarized the results of this analyses in the last column of Table 1.

It is worthwhile to note that these results cannot be conclusive although they give precise indications on the main differences between the crystal micromorphologies of biogenic and abiogenic carbonates. This means that, in order to reach meaningful conclusions, we need to analyse the morphology of other samples as well as a statistically significant number of particles. Furthermore, special attention should be given to diagenesis (Tucker and Wright, 1990), namely the chemical and physical post-depositional transformation processes, that alter the original chemistry and microstructure of the carbonate minerals, producing a uniform final product difficult to discriminate between biotic vs abiotic origin. However, we think that the effort toward this line of research may possibly provide a method useful for discriminating the origin of Martian carbonates with the aim to characterize the surface and near-subsurface of the red planet in search for extraterrestrial life.

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