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Evidence for the microbial origin of “living rocks” erratic masses

Pellegrini Marika, Mignini Amedeo, Cacchio Paola, Djebaili Rijhab, Vaccarelli Ilaria, Farda Beatrice, Ercole Claudia, Del Gallo Maddalena

Department of MeSVA, Environmental Sciences, University of L'Aquila, Italy.

Living rocks, silicate skeleton masses of quartz and feldspars merged by massive carbonate concrete, have so far been recognized as abiotic. We studied the role of the calcifying microbiota in the formation of erratic masses sampled in Romania (Trovant) and Italy (Sibari and Rome). To test *in vitro* regeneration, boxes filled with sand and with minimum mineral medium were inoculated with microbial suspensions of natural samples. Natural and *in vitro* regenerated samples were investigated by SEM-EDX (Scanning Electron Microscopy with Energy Dispersive X-ray analysis). SEM observation of natural samples revealed the presence of calcified microbial biofilms and bacterial imprints between overlapping sedimentary layers. The presence of abundant nanoforms bacteria and their biofilm and corrugated multilayer structures were showed in Rome natural samples. While in natural Sibari samples we found more elongated shape concretions. Within *in vitro* systems we observed the formation of bioliths (4-8 mm) after few weeks. SEM observation of *in vitro* regenerated bioliths showed sandy aggregates, with multilayered structures and biofilm interposition. The microanalysis allowed us to identify carbonate sediments in both natural and regenerated samples, and carbonate bridges between silica particles of the *in vitro* samples. The successful *in vitro* regeneration and SEM-EDX findings showed that calcifying microorganisms contributed to the erratic masses’ formation. The plausible mechanisms involve CaCO3 biomineralization by bridges and/or sedimentary layers formation. Extracellular polymeric substances (EPS) and diffusible proteins production can also lead to the formation of $CaCO_3$ concretions. The latter was more evident in Sibari concretions; the elongated concretions, formed by precipitation of $CaCO_3$ crystals far from colonies, are promoted by EPS and diffusible proteins. The presence in Rome samples of numerous clusters of putative hydroxyapatites (with different sizes), fasciculated calcium carbonate crystals, nanobacteria cells and biofilms suggested a more complex biotic mechanism than Trovant and Sibari concretions.