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## Isolation and characterization of calcifying bacteria from “living rocks”: a possible carbon sink

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CO<sub>2</sub> emissions triggered by anthropogenic and natural activities contribute to climate change, one of the current environmental threats of public and scientific concern. At present, microbially-induced biomineralization of CO<sub>2</sub> by calcium carbonate (CaCO<sub>3</sub>) is one of the highly topical study subjects as carbon stabilization process. In the present study we focused our attention on the calcifying bacteria of “living rocks”. The origin of these concretions, composed by a silicate skeleton of quartz and feldspars, merged by massive carbonate concrete, has so far been recognized as abiotic. Within this study we investigated the role of calcifying bacteria in their formation of these concretions and we isolated and characterized the species with CaCO<sub>3</sub> precipitation abilities. Concretions were sampled in Romania (Trovan) and Italy (Sibari and Rome). Samples were first analyzed for their culturable microflora (i.e. isolation, CaCO<sub>3</sub> precipitation capability and molecular characterization). Then, *in vitro* regeneration tests were carried out to confirm the contribution of bacteria in the formation of these erratic masses. Moreover, natural samples and bioliths regenerated *in vitro* were (i) observed and analyzed by scanning electron microscopy (SEM-EDS) and (ii) characterized at molecular level by DNA extraction and 16S rRNA analysis (V3-V4 regions). By isolating and characterizing the culturable microflora, we obtained 19 calcifying isolates, with different morphological, bacteriological and mineral precipitation properties. These evidences have given a first relevant contribution for the definition of the biotic role to the formation of these concretions. These evidences were confirmed by the efficient *in vitro* regeneration and SEM-EDS analysis. The molecular identification of the isolates and the comparison of the data obtained from the Illumina sequencing with those present in the literature, allowed us to hypothesize the genera that most likely contributed to the formation of these concretions. The results obtained provide a good scientific basis for further studies, which should be directed towards the use of isolates in studies of environmental and socio-economic relevance. Several studies demonstrate that microbially mediated biomineralization has the potential to capture and sequester carbon. Calcium carbonate, is a stable pool of carbon and is an effective sealant to prevent CO<sub>2</sub> release back into the atmosphere.