## Carbonate biomineralization under aerobic and anaerobic conditions by a novel deep subsurface bacterial isolate

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The Iberian Pyritic Belt (IPB), one of the largest massive sulphide deposits on the world, is considered to be a Martian analogue.

A recent study [1] has shown the occurrence of microscale iron bearing carbonate minerals in sediments from the acidic Tinto River (IPB, Huelva, Spain) as well as in its subsurface. Even though microbial agents responsible for iron and carbonate precipitation in the river sediments are being identified, the specifics on subsurface biomineralization in Martian analogue environments are still poorly understood.

In order to further elucidate this and other open questions, the Iberian Pyritic Belt Subsurface Life (IPBSL) project is currently in progress. In that context, two boreholes (BH11 and BH10) of 339m and 613m depth were drilled into the IPB subsurface.

In this study we report the ability of the novel actinobacteria isolate NR2A-C7, obtained from a 297m deep sample from the IPBSL BH11, to mediate the precipitation of iron and carbonate under aerobic and anaerobic conditions. The minerals formed included crystalline iron phosphates and iron-calcium carbonates with similar composition to minerals identified in core samples from the same borehole.

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## Calcium carbonate precipitation by CO<sub>2</sub> uptake in alkaline solutions

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The uptake of  $CO_2$  in  $Ca^{2+}$  ion bearing alkaline solutions causing subsequent precipitation of  $CaCO_3$  is valid for many natural and man-made environments, e.g. formation of travertine and scaling [1, 2]. However, significant gaps of knowledge exist in respect to combined  $CO_2$  uptake and  $CaCO_3$  precipitation kinetics.

In the present study  $CO_2$  uptake and  $CaCO_3$  precipitation mechanisms and rates were experimentally studied by diffusion of  $CO_2$  through a polyethylene membrane (0.2 mm) from an inner to an outer solution containing 10 mM of  $CaCl_2$ (25°C). The pH was kept constant during two analogous sets of experiments at 8.3, 9.0, 10.0 11.0 or 11.5 by titration using 500 mM NaOH solution.

By exceeding a supersaturation threshold CaCO<sub>3</sub> is formed in the outer solution. (Micro)Raman and XRD pattern as well as SEM images clearly reveal the formation of calcite and vaterite at all pH values, whereas at  $pH \ge 10.0$  aragonite additionally occurs. NaOH titration curves and [Ca2+] reflect  $CO_2$  uptake rates ( $\propto$  ACAR: aqueous  $CO_3^{2-}$  accumulation rate [3]) and precipitation rates of CaCO<sub>3</sub> (R). At elevated pH of the outer solution the ACAR is significantly higher and less time for nucleation of CaCO3, t<sub>fc</sub>, is required compared to lower pH conditions (e.g. pH 8.3 and 10.0 result in ACAR = 10 and 122  $\mu$ M h<sup>-1</sup> l<sup>-1</sup> and t<sub>fc</sub> = 7.8 and 1.6 h, respectively). At the given total experimental time of 20 h the amount of precipitated CaCO<sub>3</sub> was similar for all 10 experiments. This can be explained by the significantly higher R values subsequent to nucleation at low versus high pH (e.g. pH 8.3 and 10.0 with R = 645 and 178  $\mu$ M h<sup>-1</sup> l<sup>-1</sup>, respectively) which correlates with a general decrease of  $[>CaHCO_3^0]$  at the CaCO3 surface [4]. Finally, the R values decrease to a nearly constant value of 93  $\pm 10 \ \mu$ mol h<sup>-1</sup> l<sup>-1</sup> in all experiments.

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