Silicon within fossil and cultivated coccoliths of *Helicosphaera carteri*: new insights from X-ray fluorescence and infrared spectroscopy analyses

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Coccolithophores, one of the main marine calcifiers, significantly impact the atmosphere-ocean CO_2 exchanges and the global carbon cycle since geological time by capturing CO_2 through photosynthesis and permanently fixing carbon in their coccospheres composed of micrometrical carbonate plates, i.e. coccoliths. However, the physiology and proliferation strategies of coccolithophores are still poorly known, especially at species-specific level. Recent studies on DNA sequences proved that some living species need silica-transporters (SIT) or silica-like transporters (SILT) to build coccoliths. To date SIT and SILT have been identified only in few species such as *Coccolithus braarudii*, *Calcidiscus leptoporus*, *Prymnesium neolepis,* and *Scyphosphaera apsteinii*; whereas the species belonging to the Isochrysidales, such as *Emiliania huxleyi* and *Gephyrocapsa oceanica*, do not record the presence neither of SIT or SILT.

A deeper knowledge on Si-requiring species is important to understand their physiology and distribution, as well as the evolutionary steps driving the SIT and SILT strategy. To investigate silicon presence within coccoliths, we analyzed for the first time both cultured and fossil coccoliths at three beamlines of Elettra Sincrotrone Trieste: i) Hard X-ray Fluorescence (XRF), ii) Soft X-ray Microscopy and Low Energy X-ray Fluorescence (TwinMic), and iii) Infrared Spectroscopy (SISSI). We selected the species *Helicosphaera carteri* since: a) it is heavily-calcified, b) its big-sized coccoliths make the picking of fossil samples wieldier, c) it belongs to the Zygodiscales family which comprises one species containing SILT. Species-specific fossil coccoliths were picked with a micromanipulator from two deep-sea sediment samples of the NW Pacific (Ocean Drilling Program Site 1209)

and deposited during Marine Isotope Stage (MIS) 5, which is considered a good analogue of modern warming, and during the foregoing glacial phase MIS 6. To dispel any bias derived from fossilization processes, we also analyzed single coccoliths extracted from monospecific cultures of *H. carteri* grown under 290 ppm of CO_2 mimicking the conditions recorded during MIS 5.

Thanks to these newly applied methodologies, we present preliminary results showing the Si presence within both cultured and fossil *H. carteri* coccoliths. More specifically, XRF beamline detected Si in cultured sample at macroscale, whereas TwinMic microscope returned Si distribution maps on single coccoliths at sub-micrometric length scale. SISSI beamline provided semi-quantitative data on $CaCO_3$ and Si content. Combining these multi-beamline and multi-technique data, we documented higher Si content in the cultured samples than in the fossil ones, possibly due to preservation issues. Our data revealed the potentiality of XRF, TwinMic and SISSI beamlines in analyzing the chemical composition of inspected samples, both at elemental and molecular scale, within both cultured and fossil coccoliths at species-specific level. This is pivotal in coccolithophore studies since monospecific elemental analyses in the fossil record are extremely complex, if not almost impossible. This research was funded through MUR for ECORD-IODP Italia.