The uncultivated side of coccolithophores: Digging into the organic matter

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The calcium carbonate production from coccolithophores has been widely studied because of its important contribution to global biogeochemical cycles and long-term carbon fluxes. On the other hand, the organic fraction of coccolithophores is still poorly studied, despite its importance in carbon transfer among different oceanic reservoirs. To date, only the species *Emiliania huxleyi* has been studied in detail for its organic composition (Aveiro et al., 2020): >50% $CaCO_3$, 20% lipids, 15% proteins, and 4% carbohydrates. Specific studies on other species of coccolithophores are necessary to differentiate their organic fraction production.

To increase the knowledge of the organic compounds involved in coccolithophore physiology, *Helicosphaera carteri* was chosen as the target species. The analysis was carried out at a lower resolution with easily accessible instruments (e.g., benchtop Fourier Transform Infrared (FTIR) Spectroscope). This species' relatively large cell size (15 µm), as compared to *E. huxleyi*, makes possible a more detailed analysis at the single cell level, exploiting the higher brightness of the synchrotron source. Here, the IR spectra obtained from both entire coccospheres and decalcified cells are compared to data (10 µm spatial resolution) that were collected at the Synchrotron Infrared Source for Spectroscopy and Imaging (SISSI-Bio) beamline at Elettra Sincrotrone Trieste (Italy), during the beamtime #20235227, on single naked cells and coccoliths detached through both mechanical and chemical treatments.

The data acquired on entire coccospheres (3 replicates) with laboratory FTIR were recorded using only 5 mg of freeze-dried residue of *H. carteri*. At first, we detected weak peaks of lipids, proteins, carbohydrates, and nucleic acids because the presence of $CaCO_3$, which returned a strong signal at ~1410/cm, masked the peaks of the organic compounds. After a HCl 1M treatment, the spectra at FTIR on decalcified cells documented the presence of stronger peaks of the above-mentioned organic compounds and returned a clearer overview of the organic composition. The analysis at SISSI-Bio was performed on decalcified single cells stored in acid Lugol's solution and single coccoliths of *H. carteri.* We analyzed 100 naked cells and coccoliths that were both mechanically and chemically separated for a total of 300 different spectra. The data showed a clearer signal of the individual organic macromolecules such as proteins, polysaccharides, and lipids compared to the previous FTIR data. Moreover, at the SISSI-Bio, the analysis of organic matter on individual coccoliths obtained by mechanical separation documented the occurrence of weak signals of polysaccharides and proteins, in contrast to those chemically treated with Triton-X, bleach, and hydrogen peroxide, where only the peaks of $CaCO_3$ are evident. Multivariate analysis will be used to highlight more subtle differences between the treatments and provide more detail on protein conformation and lipid order.

In conclusion, our work draws attention once again to the importance of sample preparation and documents the pos-

sibility of performing FTIR analyses in the laboratory with good results even when having microquantities of residue available. Moreover, our data underline the importance of using innovative, high-resolution techniques for a better characterization of the organic matter produced by coccolithophores during their life cycle. This is critical for a better understanding of their physiology and thus the role that different organic compounds might play in coccolithogenesis and biocalcification.

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References:

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