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Methods for Transmission Electron Microscope (TEM) and Electron Energy Loss Spectroscopy (EELS) investigations

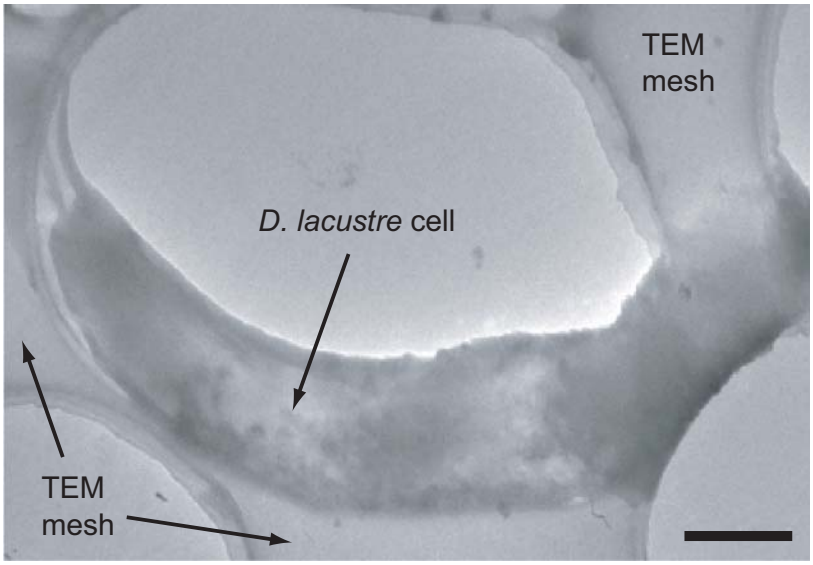
The microbial carbonate precipitate was subsampled for TEM observations through agitation and resuspension of the carbonate floccules. Subsamples were obtained with a sterilized, N_2-flushed syringe. A drop of the resuspended floccules was placed on a TEM sample holder which was introduced in a conventional Topcon 002B TEM and a dedicated scanning VG HB05 TEM for observation.

EELS was used to investigate the chemistry of the microbial carbonate precipitate. EELS provides information on the chemical environment surrounding carbon atoms. It is particularly adapted for this kind of study because it discriminates between the chemical bonds formed by carbon atoms in organic matter and those of the carbonate ion, with nanometric spatial resolution. Electron energy loss spectra for P, C, Ca and O atoms were obtained in both microscopes using a CCD based Gatan spectrometer. Particular attention was paid to minimise the degradation due to electron irradiation of the analyzed material. TEM -EELS experiments were carried out at 100 and 200 keV using different probe intensities. The EELS data have been acquired using an energy dispersion of 0.5 eV per channel and an energy resolution of around 1.5 eV. The occurrence of carbonate ions in the materials analyzed with EELS was determined based on EELS spectra published by Garvie et al. (1994) and on an EELS experiment that we carried out on reference calcite. EELS chemical analyses have been obtained using a conventional AE^{-r} law edges extraction and tabulated Hatree-Slater cross sections (Egerton, 1996). The approximate stoichiometry of the material contained in the globules was determined with the spectrum-imaging technique (Jeanguillaume and Colliex, 1989).

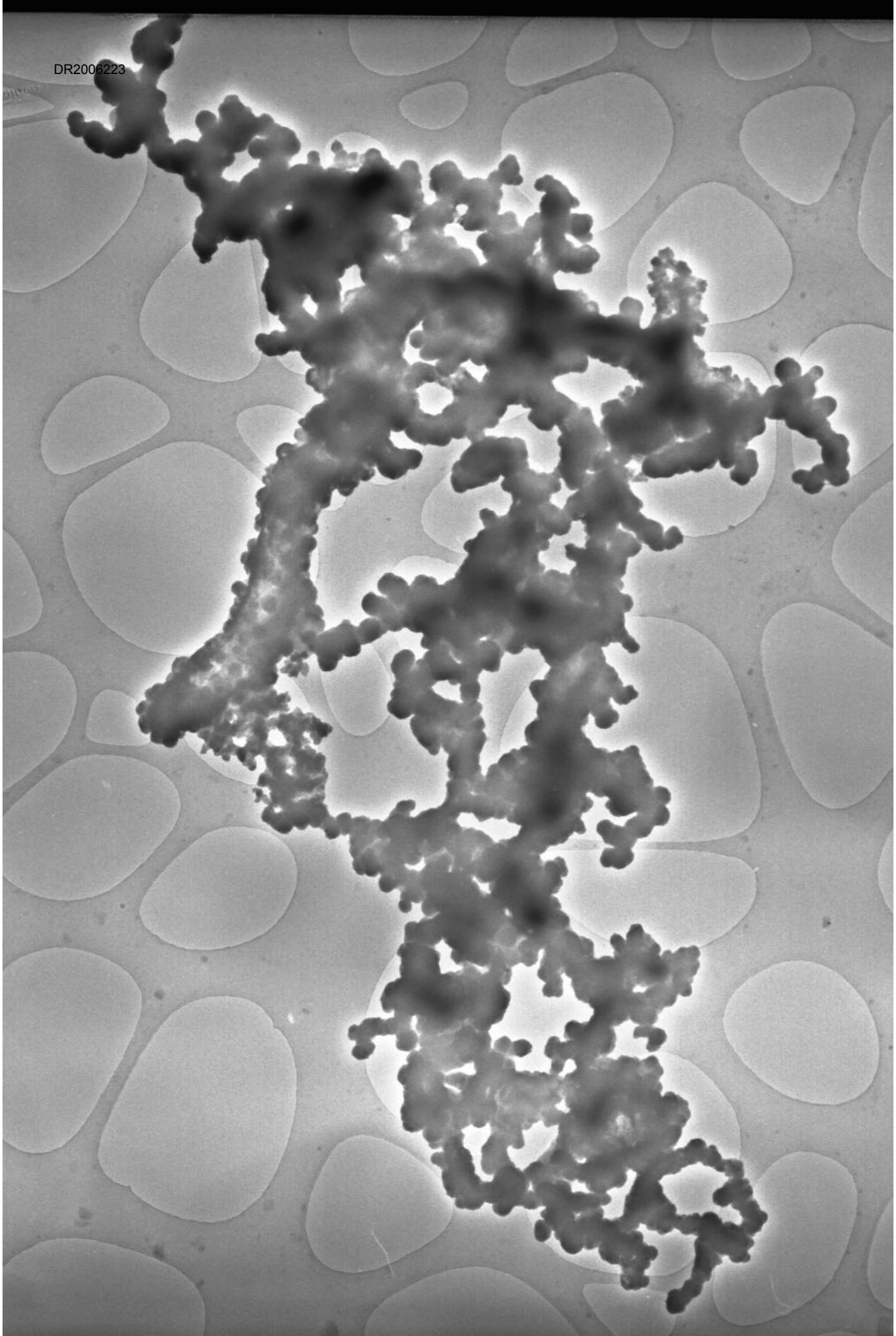
Egerton, R.F., 1996, Electron energy loss spectroscopy in the electron microscope (second edition): New York, London, Plenum Press, 500 p.

Jeanguillaume, C., and Colliex, C., 1989, Spectrum-imaging: The next step in EELS digital acquisition and processing: Ultramicroscopy, v. 28, p. 252-257, doi: 10.1016/0304-3991(89)90304-5.

Data Repository Item



Supplementary Figure 1 - Transmission Electron Microscope image of a *D. lacustre* cell in the DSMZ nr. 813 culture medium (scale bar: 500 nm). No globule-like structures have been observed in these cultures. Spots of electron-dense material on the cell wall probably consist of EPS. Their abundance is negligible compared to the abundance of globules observed in the precipitation experiment with *D. lacustre.*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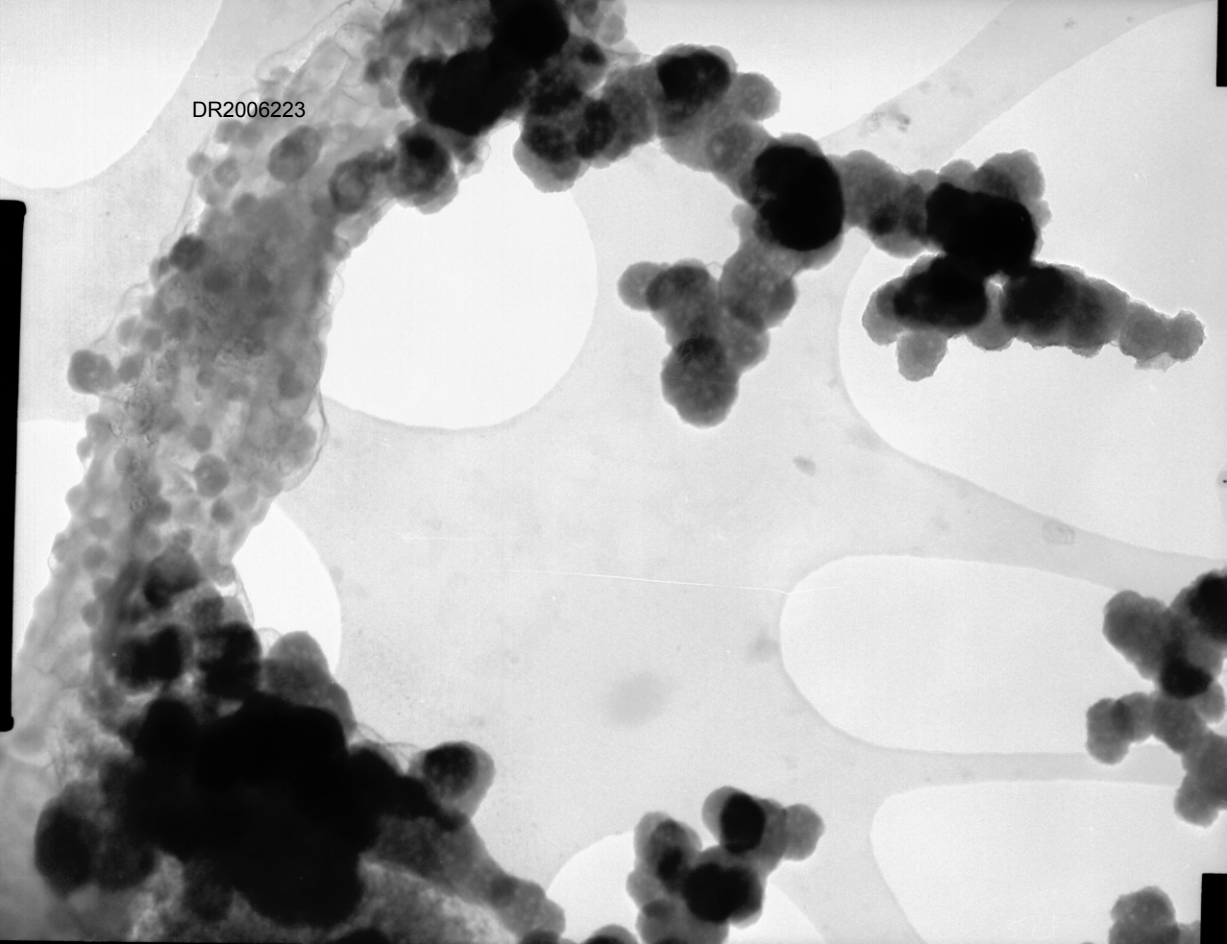
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