

**Fig. S1.** Electron microscopy images and overlap of C (blue), Ca (green), and P (red) elemental maps obtained by energy dispersive X-ray spectrometry (EDXS) mapping of Pasteur Culture Collection (PCC) strains that do not form intracellular carbonates. Images and EDXS maps do not always correspond to the same area. Images were acquired by SEM in backscattered electron mode (AsB detector). EDXS mapping was performed by SEM for most cases, except for PCC 7421, 6307, 73106, 10914, 9431, 7107, and 7202, for which we show scanning transmission electron microscopy (STEM)-EDXS maps.

[Fig. S1](#)

**Fig. S2.** Representative EDXS spectra of Mg- and K-rich PolyP granules in PCC 7421, Mg-, K-, and Ca-rich PolyP granules in PCC 7107, Ca-carbonate inclusions in PCC 6312 and PCC 7203, and representative selected aperture electron diffraction (SAED) patterns of Ca-carbonate inclusions in PCC 6312. (*Left*) SAED pattern was zero-loss filtered. (*Right*) SAED patterns were not zero-loss filtered. All electron diffraction patterns show diffuse rings indicating that Ca-carbonate inclusions are poorly crystalline. Scale bar is  $3 \text{ nm}^{-1}$  for all of the patterns. The major ring is at  $\sim 3.39 \text{ \AA}$ , which might be consistent with (111) lattice planes of aragonite.

[Fig. S2](#)

**Fig. S3.** Scanning transmission X-ray microscopy analysis of carbonate inclusions in PCC 6312, *Candidatus* Synechococcus calcipolaris G9, PCC 6716, PCC 6717, and PCC 7425. Maps of carbonate inclusions (red), polyphosphate inclusions (green), and the proteins in cells (blue) were obtained based on the difference in their X-ray absorption near-edge structure (XANES) spectra at the C K-edge (280–320 eV) and the Ca  $L_{2,3}$ -edges between 345 and 360 eV (1). XANES spectra measured on the carbonate inclusions are also shown. The peak at 290.2 eV (arrow) is characteristic of C  $1s \rightarrow \pi^*$  electronic transition in carbonate functional groups. The peak at 300.3 eV is characteristic of C  $1s \rightarrow \sigma^*$  electronic transition in carbonate functional groups.

1. Cosmidis J, Benzerara K (2014) Soft X-ray scanning transmission micro-spectroscopy. *Handbook of Biomineralization*, eds Gower L, DiMasi E (Taylor & Francis, London).

[Fig. S3](#)

**Fig. S4.** SEM analyses of *Candidatus* Gloeomargarita lithophora D10 cultured in different culture media. Cultures were transferred and grown three times in the mentioned growth media before SEM observations. Calcium is in green and phosphorus in red in SEM-EDXS maps. Cells systematically show the presence of PolyP granules and  $\text{CaCO}_3$  inclusions regardless of the culture medium.

[Fig. S4](#)

**Fig. S5.** SEM analyses of *Candidatus* Synechococcus calcipolaris G9 cultured in ASNIII-vitB12, BG11<sub>o</sub>-10%BG11-NaHCO<sub>3</sub> and BG11<sub>o</sub>-10%BG11. Cultures were transferred and grown three times in the mentioned growth media before SEM observations. Calcium is in green and phosphorus in red in SEM-EDXS maps. *Ca. S. calcipolaris* G9 cells systematically show Ca-carbonate inclusions at the poles of the cells regardless of the culture medium. (Scale bars: 2  $\mu\text{m}$ .)

[Fig. S5](#)