



Figure 8 (a) CLSM image of LR-white-embedded, calcein- and DAPI-stained microbialite from 10 cm water depth obtained by a concomitant excitation at 405, 488 and 543 nm, showing fossilized Pleurocapsales cells. (b) Superimposed composite tricolor Raman map displaying the spatial distribution of aragonite (red), hydromagnesite (green) and LR-white resin (blue).

from the cell wall) occupies the old cytoplasmic space (Couradeau *et al.*, 2013).

In summary, Pleurocapsales seem to drive the specific precipitation of aragonite in the presence of natural, high Mg^{2+} concentrations via the concomitant photosynthesis-derived alkalization of their local environment and the specific accumulation of Ca^{2+} in the F-layer of vegetative cells. In doing so, they become encrusted and progressively fossilize (Couradeau *et al.*, 2013). At the same time, increasing carbonate precipitation prevents access to light. Progressive encrustation and increasingly limited access to light may be seen as important selective forces for the evolution of baeocytes. Motile baeocytes, lacking an F-layer, may migrate towards softer, non-encrusted parts of the biofilm where light intensity is more favorable for photosynthesis, thus escaping from complete encrustation in aragonite and allowing the continuous development of the colony. Indeed, we observed that baeocytes were mostly located towards the external part of the biofilm, whereas encrusted cells were towards the interior (Figures 2a and b, and Figure 5).

For a long time, one of the most important questions in stromatolite formation has been (and still is) whether (i) microorganisms develop first and induce carbonate precipitation (directly or indirectly) or whether (ii) carbonate precipitation occurs abiotically and microbes colonize and/or entrap and consolidate the precipitates. Although the two things likely happen *in vivo*, the Pleurocapsales example is a clear case of carbonates formed by microorganisms.

Members of the Pleurocapsales not only seem to be specific active factors in mineral formation in the Alchichica microbialites, as shown by coupled CSLM and Raman spectroscopy, but are also quantitatively abundant in Alchichica and widespread in other microbialite systems. Thus, Pleurocapsales were detected in all microbialite fragments collected in

the Alchichica Lake, being dominant at greater depths (Couradeau *et al.*, 2011). Furthermore, phylotypes of Pleurocapsales related to those identified in Alchichica microbialites have been detected in numerous other microbialite systems, such as in Lake Van, Turkey (López-García *et al.*, 2005), Lake Satonda, Indonesia (Kempe and Kaźmierczak, 1993), Shark Bay, Australia (Goh *et al.*, 2009) and the Bahamian stromatolites (Foster *et al.*, 2009). Therefore, Pleurocapsales, being abundant, closely associated with the mineral phase and actively promoting mineral precipitation, seem particularly well adapted to microbialite ecosystems and have an important role as ecological architects of microbialite structures.

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