

Protist diversity

Although protists are conspicuous microbialite inhabitants [57], their diversity in these environments has been rarely studied. To prevent library saturation with animal sequences, we amplified SSU rRNA genes using the primer UNonMet, biased towards non-metazoan eukaryotes [58]. In addition to libraries from the selected samples AL31, AL67, AL52 and aquarium microbialites, we amplified protist SSU rDNAs from the aquarium plankton (AQ1w and AQ2w) and non-calcified biofilms growing on aquarium walls (AQ1b and AQ2b). These samples should serve as controls to identify specific protist phylotypes associated with growing microbialites. The number of clones analyzed for each sample is summarized in Table 2.

There were important differences between field and aquarium samples and also between plankton and microbialites in the aquaria, whereas the aquarium non-calcified biofilms were similar to the aquarium microbialites (Figure 3). Field microbialites were dominated by one single chlorophyte (ChlorophytOTU05, related to the sessile genera *Pseudendoconium* and *Blidingia*), representing ~90% of all sequences in AL31 and AL67, and ~70% in AL52 (Figure 5). Two additional chlorophytes were identified in AL52: ChlorophytOTU06, also related to those two genera, and ChlorophytOTU01, very close to *Rhizoclonium hieroglyphicum*, an entangling filamentous algae widespread in microbial mats in fresh or brackish waters [59]. AL52 also contained a dinoflagellate OTU related to the photosynthetic genus *Woloszynskia*. No other photosynthetic eukaryotes were found in the lake, although they certainly exist since living diatoms were observed by microscopy (Figure 2) and their chloroplast SSU rRNA genes were detected in sample AL31 (see above). Field samples were thus dominated by green algae, which possibly masked other eukaryotes present in minor proportions. Thus, only two additional non-photosynthetic phylotypes were identified in AL31, both corresponding to fungi (Figure S13).

Aquaria samples were far more diverse. Among photosynthetic protists, ChlorophytOTU05, dominant in field microbialites, was also abundant in aquarium microbialites, especially AQ1. However, it was absent from both the aquarium plankton and the non-calcified biofilms (Figure 5). It thus seems specifically associated to microbialites, opening the possibility that it plays a role in their formation or stability. A few other chlorophytes and several other photosynthetic lineages were identified in aquaria, notably diatoms (StramenoOTU05-07) and chrysophytes (StramenoOTU03, frequent in plankton). Concerning heterotrophic eukaryotes, ciliates (Figure 5) and very diverse opisthokonts were found in the aquaria (Figure S13). The latter included most notably Fungi, with typical Ascomycota, Basidiomycota and Chytridiomycota, but also OTUs of the environmental LKM11 group, now classified as Rozellida or Cryptomycota [60]. A relatively large diversity of Amoebozoa and choanoflagellates was also found, the latter almost exclusively in AQ1 and never in the planktonic fraction. We also identified nucleariids and several divergent sequences at the base of the Choanoflagellida/Icthyosporea and at the base of the Metazoa without close relatives (Figure S13).

Discussion

To address the long-term question of understanding microbial-mineral interactions and how microbialites form, we first aimed at characterizing microbial communities inhabiting Alchichica microbialites at different depths. The recurrent presence of particular abundant lineages may point out to specific metabolisms and lead to hypotheses about their role in carbonate precipitation and

microbialite formation. Another important issue is the possibility to preserve a significant fraction of the original microbial communities in laboratory aquaria. This would allow mineralization experiments under controlled conditions using complex and fairly genuine diverse microbial communities. Thus, we studied the diversity of microorganisms belonging to the three domains of life in an integrative approach rarely undertaken for this kind of systems.

Alchichica field microbialite community structure and its variation with depth

Field microbialites at all depths were largely dominated by Cyanobacteria and Alphaproteobacteria. As in Shark Bay stromatolites, where ~10% of the Alphaproteobacteria were potential anoxygenic photosynthesizers [29], many Alchichica Alphaproteobacteria are likely photosynthetic. Most likely, Alchichica alphaproteobacterial phylotypes display diverse metabolisms going from autotrophy to heterotrophy which, together with their richness, suggests an important role in microbialite biofilm organization and activity. Chloroflexi, present in all samples and probably abundant according to DGGE fingerprinting, was the third Alchichica bacterial group with photosynthetic members. In addition to photosynthesizers, typical heterotrophs such as Planctomycetales, Bacteroidetes and Actinobacteria, were recurrently present at relative high frequency, whereas Beta-, Gamma- and Deltaproteobacteria and Firmicutes showed more variable proportions (Figure 3). The dominant Cyanobacteria and Alphaproteobacteria, accompanied by relatively abundant Planctomycetales, Firmicutes and Bacteroidetes have been reported in comparable systems including Cuatro Ciénegas [21], Bahamas [34,35] and Shark Bay [29,33]. In addition, many of the closest relatives to Alchichica sequences come from alkaline systems, notably the giant microbialites of Lake Van, more similar by its physico-chemical characteristics to Alchichica microbialites than marine or hypersaline lake ones [13]. This observation was statistically confirmed by comparing the bacterial community composition of Alchichica samples with those of Shark Bay, Bahamas and Lake Van. All Alchichica samples clustered together, forming two clusters, one for lake samples, with 0.5 and 4 m depth samples more closely related, and the other for aquarium microbialites (Figure 6). From the other samples, although much more distant, Lake Van was closer to Alchichica samples than the marine stromatolites.

Two important observations can be outlined from Alchichica microbialite bacterial diversity. First, even if many photosynthetic lineages are present, the relative abundance of typical heterotrophic lineages suggests that they play an important role. Second, the most remarkable change along the depth gradient was the marked shift in the cyanobacterial community composition, dominated by filamentous Oscillatoriales in surface and intermediate depths (>90% of sequences at 0.5 and 4 m) and by Pleurocapsales in deeper samples (>80% of sequences, contributed mostly by the phylotype CyanoOTU35). This shift was detected by gene library comparison but also by sequencing intense DGGE bands (Figure S2 and Table 3). Although variation of the cyanobacterial composition at larger spatial scales (>few centimeters), as evidenced in Hamelin Pool [29,33] and Bahamas [32], cannot be discarded, the Oscillatoriales-to-Pleurocapsales dominance transition with depth in Alchichica is likely related to adaptation to depth and light intensity. Oscillatoriales are indeed adapted to high light intensity [61], whereas Pleurocapsales actively search low light (Waterbury and Stanier, 1978). This correlates with microscopy observations showing that filamentous Oscillatoriales tend to grow at the microbialite surface (e.g.