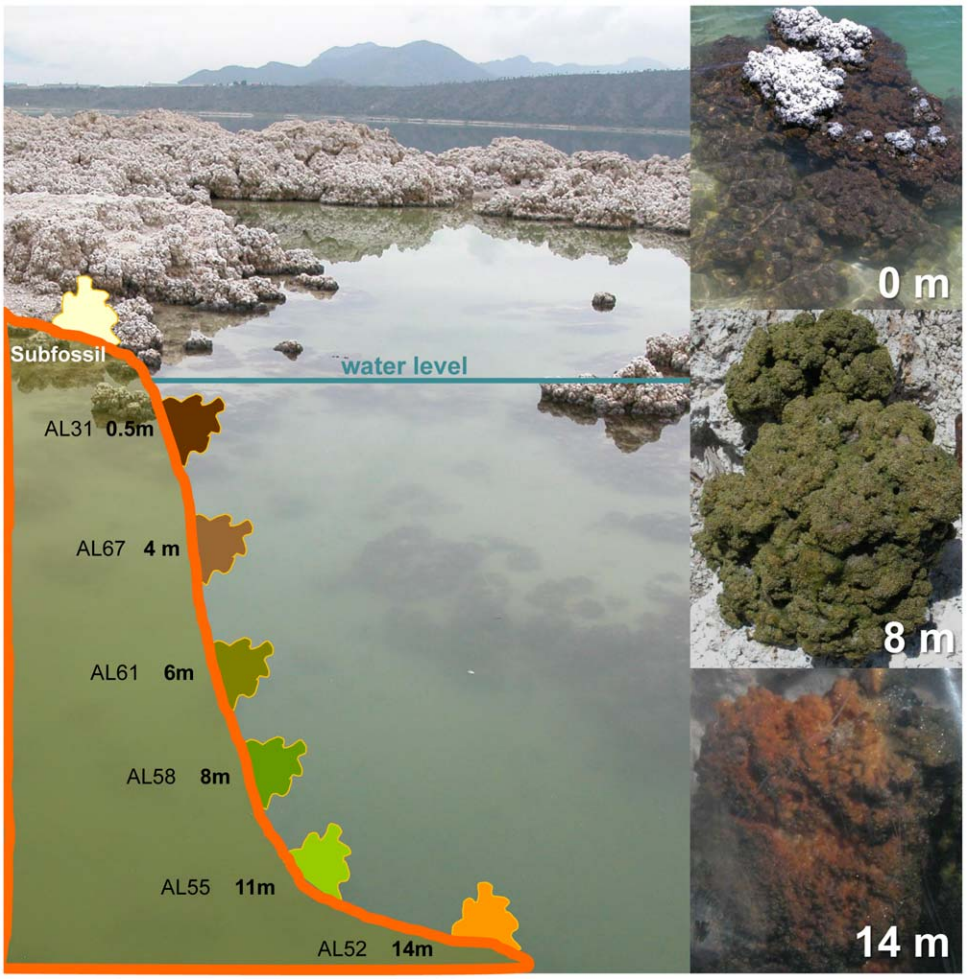

Figure 1. View of Alchichica and schematic depth profile showing the different sampling depths in the lake. Stromatolite fragments from three different depths and colors are shown on the right.
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Overview of bacterial diversity in Alchichica microbialites

Bacterial diversity in the selected samples was further characterized by SSU rRNA gene libraries (Table 2). We used general bacterial primers but also cyanobacterial-specific primers to get a finer description of the diversity within this group, since cyanobacteria usually dominate stromatolite microbial biomass, including Alchichica microbialites (Figure 2) [40], and likely play a major role in carbonate precipitation. In addition, since cyanobacterial EPS sheaths may decrease DNA extraction yield [44,45,46], using specific primers would help to detect underrepresented species. To further limit biases, we generated two bacterial and two cyanobacterial SSU rDNA libraries for each sample, except in cases when a single library allowed a coverage >80% and a small number of singletons (Table 2). There were only minor differences in the diversity obtained between the two libraries for each sample, mostly in relative proportions, in particular for a few cyanobacterial, alpha- and beta-proteobacterial phylotypes. The only significant difference was the presence of Firmicutes only in bacterial library 2. These differences are likely due to local heterogeneities and/or to a different coverage achieved by the libraries. However, despite these relatively minor differences, there was a rather good agreement in the bacterial diversity identified in the two libraries, which can therefore be considered as replicates. This was also the case for the most abundant cyanobacterial groups in both general and specific libraries (Figure 3). Therefore, for each sample we compiled the diversity from the two independent libraries for further inter-sample comparison.

Figure 3 shows the taxonomic distribution of bacterial clones in lake and aquarium samples. We identified members of 14 phyla and 7 candidate divisions. Remarkably, bacterial diversity was generally higher in aquaria than in field samples in terms of high-rank taxa, in agreement with the DGGE analysis, which showed more bands in the aquarium profiles (Figure S2). At phylum level, AQ1 taxa resembled those of field microbialites, especially those collected at higher depth. They were all dominated by Cyanobacteria and the Alpha subdivision of the Proteobacteria (60 to 75% of the total bacterial SSU rDNAs in field sample libraries, Figure 3). AQ2 displayed similar taxonomic composition, but Cyanobacteria and Alphaproteobacteria accounted for only ~15% of sequences. In contrast, Firmicutes, minor components in the other libraries (0 to 2%), were dominant in AQ2 (29%). The rest of bacterial taxa had variable relative proportions, probably reflecting local spatial heterogeneities and/or depth-related adaptation. For example, Betaproteobacteria represented 19% of sequences in AL67 but less than 2% in other samples. The proportion of Actinobacteria increased with depth (from 1% to 10%) whereas Bacteroidetes showed the opposite trend (from 4%