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Rapid formation of mature microbialites in Lake Alchichica, Mexico

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Running title: Rapid microbialite formation in Lake Alchichica

Originality-Significance Statement

Microbialites are rocks formed by the activity of complex microbial communities that sequester CO₂ in the form of biomass and mineral carbonates. However, little is known about microbialite growth rate and the steps that take place early during their formation. Here, we show that nascent, mm-sized microbialites form on Nylon mesh after two years of exposure in the alkaline Lake Alchichica, which harbors extensive hydromagnesite and aragonite microbialites. The associated prokaryotic and eukaryotic microbial communities were not significantly different of those associated to mature microbialite of the lake. However, only thin microbial biofilms developed on aragonite and hydromagnesite crystals, despite the fact that their microbial communities were similar to those associated to mature microbialites. Our results show that mature microbialites can form very rapidly (up to 1 mm/year) on some surfaces but not on others and open intriguing questions about carbonate nucleation that deserve future exploration.

Microbialites are emblematic sedimentary rocks formed by phylogenetically and metabolically complex microbial communities thriving under specific physicochemical conditions. Most microbialites are photosynthesis-based ecosystems frequently formed by carbonates, thereby capturing inorganic carbon in the form of both, organic matter and mineral precipitates. However, little is known about the amount of sequestered carbon and the kinetics of the process, i.e. microbialite growth rate. To assess microbialite growth rate and the influence of substrates on carbonate formation in Alchichica, an alkaline crater lake harboring well-developed carbonate microbialites, we incubated in situ sterilized Nylon mesh, hydromagnesite and aragonite crystals, and bleached-coral aragonite for two years. We observed the rapid formation of nascent hydromagnesite and aragonite-containing microbialites on Nylon mesh, with an average growth rate of ~0.6 (and up to 1) mm/year. By contrast, only thin (<0.2 mm) biofilms developed on exposed hydromagnesite and aragonite crystals and bleached-coral aragonite, suggesting decoupled microbial colonization and biomineralization and/or potential interference of those mineral surfaces with new carbonate nucleation. Microbial communities associated with 2-year-old microbialites and biofilms were fully comparable to mature communities populating Lake Alchichica indigenous microbialites.

Keywords: 16S/18S rRNA metabarcoding; stromatolite; carbonate precipitation; biomineralization; colonization; cyanobacteria; anoxygenic photosynthesis

Introduction

Microbialites are organosedimentary structures formed under the influence of phylogenetically and functionally diverse microbial communities in particular physicochemical environments (Riding, 2000; Dupraz and Visscher, 2005). Microbialites are not only emblematic as the oldest undisputed traces of life on Earth (~3.5 Ga-old laminated microbialites or stromatolites) (Tice and Lowe, 2004; Allwood et al., 2006) but also constitute poorly understood carbon traps (Ahrendt et al., 2014; McCutcheon et al., 2014; McCutcheon et al., 2019). Most marine and freshwater microbialites are formed by photosynthesis-based microbial communities that favor carbonate precipitation by locally increasing the alkalinity (Dupraz and Visscher, 2005; Dupraz et al., 2009) and/or upon an alkalinity threshold (Zeyen et al., 2017; Iniesto et al., 2020). Consequently, they constitute carbon reservoirs in the form of both, biomass and solid carbonates. To ascertain if microbialite systems are efficient carbon sinks, more quantitative measurements are needed. However, relatively little is known about early microbialite formation and growth rate. Short-term $^{13}\text{C}/^{14}\text{C}$ -labeling experiments suggest that carbonate precipitation occurs, albeit with low rates (0.05 mm/year) in Lake Pavilion (Brady et al., 2009), but other systems seem to have much higher rates, in the 0.2-0.6 mm/year range (Chivas et al., 1990; Carvalho et al., 2017).

Recent comparative studies in lakes from the Trans-Mexican volcanic belt showed that microbialite size and community structure significantly correlate with alkalinity (Zeyen et al., 2017; Iniesto et al., 2020), suggesting that local biotic and abiotic factors result in different growth rates. In particular, Lake Alchichica, an alkaline ($\text{pH} \sim 9$; $[\text{HCO}_3^-] \sim 40 \text{ mM}$) and relatively Mg-rich ($[\text{Mg}^{2+}] \sim 17 \text{ mM}$) crater lake located at high altitude (2,300 m above sea level) displays massive (meter-sized) microbialites, mostly non-laminated (thrombolites), composed of hydromagnesite ($\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 4(\text{H}_2\text{O})$) and aragonite (CaCO_3) that precipitate in situ (Kazmierczak et al., 2011; Couradeau et al., 2013; Gerard et al., 2013). Microbial communities associated to these structures have been previously studied using 16S/18S rRNA gene metabarcoding and metagenomic approaches (Couradeau et al., 2011; Saghaï et al., 2016; Valdespino-Castillo et al., 2018). Interestingly, core microbial communities shared by microbialites across various lakes accounted for higher proportions of the total microbial community in the highest-alkalinity sites, notably Alchichica (Iniesto et al., 2020). Various indicators, such as active carbonate precipitation observed in the water column and the presence of more or less developed microbialites on plants growing on the rim of the lake as well as plastic bottles and tires abandoned on the lake shore, suggested that microbialite formation is an active and relatively fast process in Lake Alchichica. However, details about the first steps, including growth rate, of microbialite formation in this lake were missing.

Results and discussion

To evaluate the capacity of calcifying microbial communities to colonize different surfaces and form new microbialites as well as to assess microbialite growth rate, we placed sterilized crystals of hydromagnesite and aragonite as well as bleached-coral fine-grained aragonite in extensively perforated 50-ml Falcon tubes that were capped with 200 μm -pore diameter Nylon mesh pieces (Fig.1A). These colonization devices were immersed at 0.5 m depth in the North shore of Lake Alchichica from January 2012 to May 2014. Nascent microbialites and biofilms growing on the incubated substrates were then collected, as well as, for comparison, fragments from large older microbialites growing at equivalent depth (0-3 m) below the water surface on the North and West shores (Supplementary Methods, Supplementary Fig.1 and Supplementary Table 1) at sampling points previously studied (Couradeau et al., 2011; Saghaï et al., 2015; Saghaï et al., 2016; Iniesto et al., 2020).

After the 28 month-incubation period, small, distinct microbialite structures of up to approximately 2 mm in diameter could be observed growing on the Nylon mesh that was used as permeable barrier capping the Falcon tubes (Fig.1A-C). These formed discrete structures that tended to merge, and not a continuous calcifying biofilm, highlighting the importance of local nucleation. Individual measurements allowed an estimation of an average microbialite growth of ~ 0.6 mm/year (and up to 1 mm/year), although this growth rate might be different for already growing structures. No macroscopically visible stratification or lamination was observed. To determine the overall mineral composition of these incipient structures, we applied attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy (Supplementary Methods). The obtained spectra highlighted their composite nature, unambiguously showing the occurrence of two distinct carbonate phases: hydromagnesite and aragonite (Fig.1D). In addition, we detected a silica-rich phase, possibly associated with diatom frustules (ochrophytes; Fig.2C and D). This mineral composition is consistent with previous observations made on mature microbialites from this lake site (Zeyen et al., 2019), indicating that nascent microbialites have a similar overall mineral composition to well-established microbialites. Furthermore, since hydromagnesite is the carbonate mineral spontaneously precipitating in the lake (Couradeau et al., 2011; Kazmierczak et al., 2011) and aragonite has been shown to be spatially associated with particular microbial groups, notably some cyanobacteria (Gerard et al., 2013; Saghaï et al., 2015), the observed composition strongly suggests active biomineralization in nascent microbialites. By contrast, only a thin biofilm (a few tens up to 1-200 μm) but no apparent microbialitic structures formed on any of the incubated mineral fragment surfaces, whatever their orientation, suggesting that microbial colonization of surfaces can be uncoupled from biomineralization. The absence of microbialite formation on the exposed mineral surfaces is unlikely to be strongly influenced by slight differences of physicochemical conditions within the perforated Falcon tube compared to the surface of the Nylon mesh. First, mineral fragments occupied around 5% of the inner 50-ml Falcon volume and water could circulate via the many

~3-5 mm diameter holes suggesting that, if there were chemical differences between the inside and outside of the tube, these were minor. In any case, these differences would be comparable to those found between small protected pockets in the very irregular cauliflower-like native microbialites, with limited water circulation, and the open lake waters. Second, small pocket zones and irregular shapes in native growing microbialites result in the existence of areas protected from direct light incidence. This, together with the fact that microbialites actively develop from the surface of the lake to at least 40 m depth (personal observations), implies that the observed patterns cannot be easily explained by differences in light intensity and quality. Therefore, although these initial observations of biofilm-only formation on aragonite and hydromagnesite crystals after two years of exposure need to be confirmed by more extended experiments, the observed patterns might suggest some type of nucleation interference or inhibition caused by those mineral surfaces.

To determine the microbial community structure associated with these samples, we studied the prokaryotic and eukaryotic components of two nascent microbialites growing on the Nylon mesh and the 2-year-old biofilms growing on the mineral substrates by a metabarcoding approach. We amplified 16S and 18S rRNA gene fragments and massively sequenced the resulting amplicons using paired-end Illumina MiSeq. After quality trimming, we built operational taxonomic units using SWARM and Cd-hit at 97 and 98% identity thresholds, which yielded comparable prokaryotic OTU numbers (Supplementary Methods; Supplementary Table 2). We retained SWARM clustering for subsequent analyses due to the resulting lower, more tractable, number of eukaryotic OTUs. OTUs were assigned to phyla and/or high-rank taxa by sequence similarity and, for divergent OTUs, phylogenetic placement in reference phylogenetic trees (Supplementary Tables 3-4). The community structure of nascent microbialites but also of the biofilms was comparable to that of mature Alchichica microbialites in terms of relative abundance and, most especially, OTU diversity (Fig.2A-D). This was further shown by the scattering of mature and young samples in a non-metric multidimensional scaling (NMDS) ordination analysis (Fig.2E; Supplementary Fig.5). Prokaryotic communities were rich in Cyanobacteria and their associated hydrocarbon-scavenging taxa (Bacteroidetes, Planctomycetes, Verrucomicrobia). Anoxygenic photosynthetic bacteria were also abundant, notably Chloroflexi and some Alphaproteobacteria (Rhodobacterales, Rhodospirillales), but also some Gammaproteobacteria (Chromatiaceae) and minor proportions of Chlorobi. Green algae and diatoms (ochrophytes), together with ciliate and fungi were the most relatively abundant eukaryotes. However, because they represent a minor part compared to the prokaryotic component (Saghäi et al., 2016), the relative abundance of eukaryotic taxa is likely affected by higher stochasticity. Only minor differences appeared in terms of relative abundance of major groups; the most notable being the presence of a higher proportion of the *Thermus-Deinococcus* clade in the sampled aragonite-colonizing biofilm (Fig.2A). Despite these minor differences, the two nascent microbialites and the three mineral-associated biofilms shared over 900 OTUs with mature

Alchichica samples collected at the north (Alchichica N) and west (Alchichica W) shorelines (Fig.2F). These shared OTUs represented 80-90% of the total prokaryotic OTUs, and 87-97% of the total eukaryotic OTUs, in Alchichica N and W samples, nascent microbialites and mineral-associated biofilms (Fig.2G-H; Supplementary Table 5). PERMANOVA pairwise comparisons showed that differences between mature, nascent microbialite communities or 2-year-old biofilms growing on incubated minerals were not significant or very marginally so (p-value 0.05 - 0.83; Supplementary Table 6). Biofilm and microbialite communities were very different from planktonic communities along the water column of the lake (Iniesto et al., submitted). This can be easily visualized in an NMDS plot, where microbialite-associated and planktonic communities clearly clustered apart (Supplementary Fig. 6).

Collectively, our results show a rapid colonization dynamics of certain newly available inert surfaces (Nylon mesh, but also plastic bottles or plants on site) in Lake Alchichica, with the formation of mature-like microbialites in terms of mineral and microbial community compositions in only two years. By contrast, although biofilms growing on incubated hydromagnesite and aragonite crystals, including coral aragonite, have similar microbial composition to that of mature microbialites, carbonate precipitation does not occur at visible scale. This suggests an uncoupling between community assembly and induced biomineralization on these surfaces and/or a potential interference of hydromagnesite and aragonite crystalline surfaces with microbialite carbonate nucleation, opening intriguing questions about carbonate nucleation in these natural ecosystems.

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Figure legends

Fig.1. Rapid growth of nascent microbialites in Lake Alchichica. **A**, photograph of colonization devices deposited in the North shore of Lake Alchichica in January 2012 and collected in May 2014. **B-C**, detail of the incipient microbialites growing on Nitex 200 μm -mesh sized membranes in the corresponding areas framed in (A). **D**, ATR-FTIR spectrum showing the presence of hydromagnesite and aragonite peaks.

Fig.2. Prokaryotic and eukaryotic community composition in mature and nascent microbialite samples as well as biofilms growing on 2-year exposed mineral substrates in Lake Alchichica, Mexico. **A**, histograms showing the phylogenetic diversity and relative proportion of 16S rRNA gene amplicon sequences. **B**, phylogenetic diversity and relative abundance of prokaryotic operational taxonomic units (OTUs) across phyla. **C**, phylogenetic diversity and relative proportion of 18S rRNA gene amplicon sequences. **D**, phylogenetic diversity and relative abundance of eukaryotic OTUs across phyla. Detailed histograms of the categories Cyanobacteria, Alphaproteobacteria and 'Other Bacteria' are provided in Supplementary Fig.2, those of Archaea and 'Other eukaryotes' are provided in, respectively, Supplementary Figs.3A and 4. **E**, Non-metric multidimensional scaling (NMDS) biplot of the different microbial communities associated to nascent microbialites and large mature microbialites growing on the North and West shores of Lake Alchichica, as well as 2-year old biofilms growing on coral aragonite (CA), aragonite (A) and hydromagnesite (HM). **F**, Venn diagram showing the number of OTUs shared by the different groups of microbialite and mineral-associated 2-year old biofilm samples. **G-H**, relative abundance and phylogenetic distribution of prokaryotic (G) and eukaryotic (H) core shared OTUs (as shown in F). Detailed sample descriptions are provided in Supplementary Table 1; nasc. mic., nascent microbialites.

Figure 1. Iniesto et al.

Figure 2. Iniesto et al.