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Seawater Mg/Ca controls polymorph mineralogy of microbial $CaCO_3$: A potential proxy for calcite-aragonite seas in Precambrian time

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ABSTRACT

A previously published hydrothermal brine-river water mixing model driven by ocean crust production suggests that the molar Mg/Ca ratio of seawater (mMg/Ca_{sw}) has varied significantly (~1.0–5.2) over Precambrian time, resulting in six intervals of aragonite-favouring seas (mMg/Ca_{sw} > 2) and five intervals of calcite-favouring seas (mMg/Ca_{sw} < 2) since the Late Archaean. To evaluate the viability of microbial carbonates as mineralogical proxy for Precambrian calcite-aragonite seas, calcifying microbial marine biofilms were cultured in experimental seawaters formulated over the range of Mg/Ca ratios believed to have characterized Precambrian seawater. Biofilms cultured in experimental aragonite seawater (mMg/Ca_{sw} = 5.2) precipitated primarily aragonite with lesser amounts of high-Mg calcite (mMg/$Ca_{calcite}$ = 0.16), while biofilms cultured in experimental calcite seawater (mMg/Ca_{sw} = 1.5) precipitated exclusively lower magnesian calcite (mMg/$Ca_{calcite}$ = 0.06). Furthermore, Mg/$Ca_{calcite}$ varied proportionally with Mg/Ca_{sw}. This nearly abiotic mineralogical response of the biofilm $CaCO_3$ to altered Mg/Ca_{sw} is consistent with the assertion that biofilm calcification proceeds more through the elevation of CO_3^{2-}, via metabolic removal of CO_2 and/or H^+, than through the elevation of Ca^{2+}, which would alter the Mg/Ca ratio of the biofilm's calcifying fluid causing its pattern of $CaCO_3$ polymorph precipitation (aragonite vs. calcite; Mg-incorporation in calcite) to deviate from that of abiotic calcification. If previous assertions are correct that the physicochemical properties of Precambrian seawater were such that Mg/Ca_{sw} was the primary variable influencing $CaCO_3$ polymorph mineralogy, then the observed response of the biofilms' $CaCO_3$ polymorph mineralogy to variations in Mg/Ca_{sw}, combined with the ubiquity of such microbial carbonates in Precambrian strata, suggests that the original polymorph mineralogy and Mg/$Ca_{calcite}$ of well-preserved microbial carbonates may be an archive of calcite-aragonite seas throughout Precambrian time. These results invite a systematic evaluation of microbial carbonate primary mineralogy to empirically constrain Precambrian seawater Mg/Ca.

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INTRODUCTION

A hydrothermal brine-river water mixing model (Hardie, 1996, 2003) suggests that the Mg/Ca ratio of seawater (Mg/Ca_{sw}) has oscillated between 1.0 and 5.2 since Late Archaean time (~2700 Ma; Fig. 1). The model's prediction of first-order Mg/Ca variations throughout Phanerozoic time (0–542 Ma) is generally supported by empirical observations from the geological record, including the major ion composition of fluid inclusions in primary marine halite (Lowenstein *et al.*, 2001, 2003, 2005; Horita *et al.*, 2002; Brennan *et al.*, 2004; Timofeeff *et al.*, 2006), the Mg content of fossil echinoderms (Dickson, 2002, 2004), and secular variation in the primary mineralogies of marine evaporates ($MgSO_4$, KCl; Hardie, 1996), abiotic carbonates (ooids, marine cements; Sandberg, 1983), and the skeletons of the major reef-building and sediment-producing marine organisms (Fig. 1; Stanley & Hardie, 1998, 1999; Porter, 2007). However, due to a paucity of such proxies in Precambrian deposits, a systematic empirical investigation of secular variation in Precambrian Mg/Ca_{sw} has yet to be undertaken.

Bacterial biofilms have been intimately involved in the precipitation of $CaCO_3$ on the seafloor for at least the past ~3.45 Gy (Knoll & Semikhatov, 1998; Grotzinger & Knoll, 1999; Grotzinger & James, 2000; Riding, 2000). Biofilms are highly

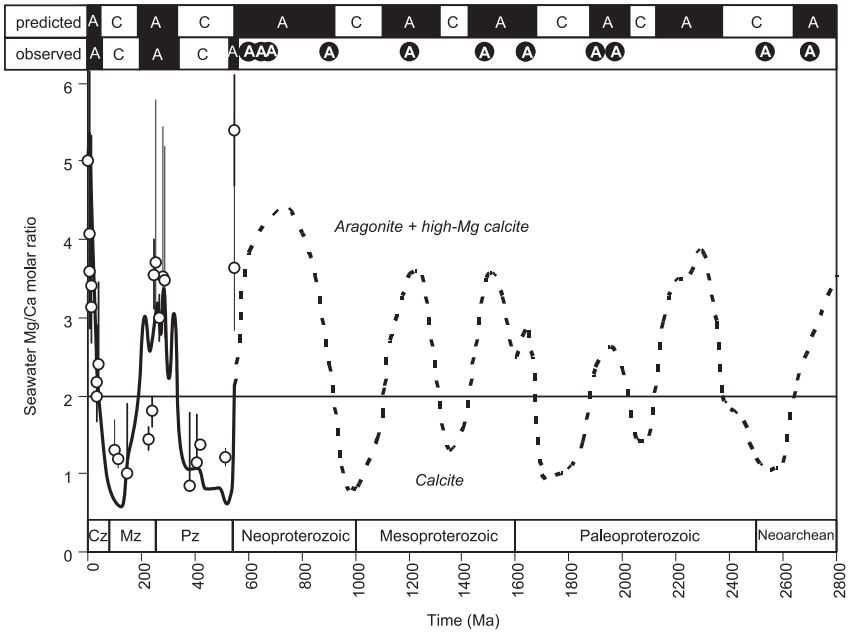

Fig. 1 Secular variation in Mg/Ca_{sw} and the original polymorph mineralogy of nonskeletal $CaCO_3$ precipitates, such as ooids, seafloor precipitates, and marine cements, since Late Archaean time. Phanerozoic (solid curve; Hardie, 1996; Demicco *et al*., 2005) and Precambrian (dashed curve; Hardie, 2003) seawater Mg/Ca is calculated from hydrothermal brine–river water mixing models driven by rates of ocean crust production (Gaffin, 1987) and granite-pluton emplacement (Engel & Engel, 1970), respectively. Open circles correspond to mMg/Ca_{sw} estimated from fluid inclusions in primary marine halites (Lowenstein *et al*., 2001, 2003, 2005; Horita *et al*., 2002; Brennan *et al*., 2004; Timofeeff *et al*., 2006). Horizontal line divides the calcite ($mMg/Ca_{sw} < 2$) and aragonite–high-Mg calcite ($mMg/Ca_{sw} > 2$) nucleation fields in seawater at 25 °C (Leitmeier, 1910, 1915; Lippman, 1960; Müller *et al*., 1972; Berner, 1975; Given & Wilkinson, 1985; Stanley & Hardie, 1999). Temporal distribution of predicted (Hardie, 1996, 2003) and observed (Sandberg, 1983) nonskeletal calcite ('C'; white rectangle) and aragonite ('A'; black rectangle) precipitates are plotted along top of figure. Closed black circles circumscribing 'A' correspond to inferred aragonitic seafloor precipitates observed in Precambrian deposits (Hardie, 2003, see references therein).

diverse bacterial communities that can precipitate $CaCO_3$ extracellularly within their micrometre-to-centimetre-thick matrices, often in association with exopolymeric substances (EPS) (Dupraz & Visscher, 2005; Altermann *et al*., 2006). Calcium carbonate precipitated within the biofilm matrix is often incorporated into the carbonate rock record, primarily as microbialites, stromatolites, and thrombolites. While the distribution and abundance of such microbial carbonates have varied significantly over geological time, most likely due to fluctuations in the $CaCO_3$ saturation state of seawater (Grotzinger & Knoll, 1999; Arp *et al*., 2001; Sumner & Grotzinger, 2004; Riding & Liang, 2005) and the evolution of grazing eukaryotes (Awramik, 1971; Riding & Liang, 2005), their associated limestones are generally well-represented from Late Archaean to Cambrian time (Riding, 2000). The relative ubiquity of microbial carbonates in Precambrian strata coupled with their largely *in situ* modes of calcification (Knoll & Semikhatov, 1998; Grotzinger & Knoll, 1999; Riding, 2000; Sumner & Grotzinger, 2004; Dupraz & Visscher, 2005; Altermann *et al*., 2006) makes them a potentially viable proxy for the composition of Precambrian seawater.

Experiments have shown that the polymorph mineralogy of $CaCO_3$ precipitated abiotically from seawater is determined primarily by Mg/Ca_{sw} ($mMg/Ca_{sw} < 2$ = calcite; $mMg/Ca_{sw} > 2$ = aragonite + high-Mg calcite; Leitmeier, 1910, 1915; Lippman, 1960; Müller *et al*., 1972; Berner, 1975; Given & Wilkinson, 1985; Stanley & Hardie, 1999), and to a lesser degree by temperature, pCO_2 (Morse *et al*., 1997), and $[SO_4^{2-}]$ (Bischoff & Fyfe, 1968; Walter, 1986), at least over the range of values that are believed to have characterized seawater since Late Archaean time (Kempe & Degens, 1985; Grotzinger & Kasting, 1993; Hardie, 1996; Morse & Mackenzie, 1998; Hardie, 2003; Kazmierczak & Kempe, 2004; Sumner & Grotzinger, 2004). Therefore, predicted variations in Precambrian Mg/Ca_{sw} (Hardie, 2003) would have subjected calcifying biofilms to a total of six intervals of aragonite-favouring seas and five intervals of calcite-favouring seas since Late Archaean time (Fig. 1). If the mineralogical response of calcified biofilms to altered Mg/Ca_{sw} in Precambrian seas mimicked abiotic calcification, then the original mineralogy of microbial carbonates may be an archive of calcite-aragonite seas throughout much of Precambrian time.

To evaluate the effect of Mg/Ca_{sw} on microbial calcification, mixed-community marine sedimentary biofilms were cultured in experimental seawaters formulated over the range of mMg/Ca ratios predicted to have occurred since Late Archaean time (1.5, 2.5, 5.2; Hardie, 2003), corresponding to a calcite sea, a boundary calcite-aragonite sea, and an aragonite sea interval.

Biofilm phylogenetic diversity, $CaCO_3$ polymorph mineralogy and distribution, and Mg-fractionation in biofilm calcite were evaluated in response to these modifications in seawater Mg/Ca.

MATERIALS AND METHODS

Specimen collection and culture

Mixed-community microbial biofilms were harvested in January 2006, from sediments in a subtidal lagoon bordering the northern coast of the Island of Roatán, Honduras. A portion of the harvested biofilms (~40 g w/wt) was rinsed for 30 s with 95% ethyl alcohol, dried at 100 °C, and stored as a control sample. The remaining living material (~500 g w/wt) was stored in plastic containers filled with natural seawater from the lagoon and transported by airplane to the laboratory at Johns Hopkins University the following day. After transport, the biofilms were placed in a holding tank of normal Mg/Ca_{sw} for 7 days. The biofilms were acclimated to the artificial seawater treatments in stages, being moved every 3 days to seawater treatments of successively lower Mg/Ca ratios, so as to minimize any chemical shock.

Approximately 20 g (w/wt) of biofilm was cultured on six glass plates (3 cm × 5 cm × 2 mm) for 120 days in each of three 38-L glass aquaria containing experimental seawaters that were identical (Bidwell & Spotte, 1985) except for their mMg/Ca_{sw}, which were formulated at 5.2 (aragonite seawater), 2.5 (calcite-aragonite boundary seawater), and 1.5 (calcite seawater). Non-biofilm control plates, identical to those on which the biofilms were grown, were placed in each of the experimental treatments throughout the duration of the experiment. The Mg/Ca ratios of the experimental seawaters were formulated with Mg^{2+} and Ca^{2+} concentrations obtained from Hardie's (1996, 2003) hydrothermal brine-river water flux model, which fixes the molar sum of these cations at the level of modern seawater. All other physiochemical parameters, including salinity (35 ppt), pH (8.2 ± 0.1), alkalinity (2.3 ± 0.1 meq L^{-1}), temperature (25 ± 1 °C), air pressure (1 atm), pCO_2 (380 ppm), and ionic strength (0.7), were fixed at normal marine values. Mg/Ca ratios remained within 5% of their initial values throughout the duration of the experiments. The aquaria were fertilized with 20.0 mg L^{-1} $NaNO_3$, 1.3 mg L^{-1} $NaH_2PO_4{\cdot}H_2O$, and 0.025 mL L^{-1} EDTA, all within the normal range for nearshore tropical marine waters (Spotte, 1979). The aquaria were maintained at 25 ± 1 °C using 50-watt electric heaters, illuminated with 10 h day^{-1} of identical irradiance (19 watts), and continuously filtered with Millennium 2000 Wet-Dry Multifilters (Aquarium systems, Inc., Mentor, OH, USA) (rate of filtration = 600 L h^{-1}).

Mineralogical and geochemical analysis

After 120 days of growth, newly grown sections of the biofilms were identified by comparison with photographs of the biofilms taken at the beginning of the experiment. The newly grown sections were removed from the seawater treatments and lightly rinsed for 30 s with 95% ethyl alcohol. The specimens were oven-dried for 24 h at 100 °C, after which their dry weight was determined. Carbon paint was used to secure ~100 mg d/wt of the biofilm specimens to cylindrical SEM carbon mounts, which were then carbon coated for 1 min. The biofilm specimens were thoroughly examined in a JEOL 8600 SEM (JEOL Ltd, Tokyo, Japan) for $CaCO_3$ precipitates. Secondary electron images of the mineral precipitates within the biofilms were obtained in the SEM.

Three 500 mg d/wt samples of newly grown biofilm material from each of the seawater treatments, as well as samples of the control biofilm, were baked in an oven at 450 °C for 4 h to combust all organic matter. The weight of the residual $CaCO_3$ material was measured and recorded as their total $CaCO_3$ content. Their percentage calcification was calculated as their total $CaCO_3$ divided by their original dry weight.

Three 300 mg d/wt samples of newly grown biofilm material, as well as samples of the control biofilm, were mixed with 95% ethyl alcohol and lightly ground for 15 s with mortar and pestle. The suspension was then injected into a 1 cm × 1 cm × 10 μm reservoir on a glass slide and allowed to dry overnight. The dried residue formed an equally thick layer of $CaCO_3$ crystals that was analysed for polymorph mineralogy using powder X-ray diffraction (XRD). The proportion of calcite to aragonite was calculated from the XRD pattern as the ratio of the area under the primary calcite peak [d(104); 3.03–3.04 Å; 2θ = 29.4–29.5°] to the area under the primary aragonite peak [d(111); 3.40 Å; 2θ = 26.2°]. The Mg/Ca ratio of the calcite precipitated within the biofilm was determined from the d-spacing of the calcite crystal lattice, which was determined by the 2θ-value of the primary calcite peak. These measurements were confirmed by EDS microprobe spot analysis in the SEM.

Clone library construction

Clone libraries were constructed to estimate bacterial diversity in biofilms cultured in the artificial aragonite (mMg/Ca_{sw} = 5.2) and calcite (mMg/Ca_{sw} = 1.5) seawaters, as well as in a control biofilm that was not subjected to the experimental seawaters, using the method described by Montalvo *et al.* (2005). Five grams w/wt material was initially harvested from the newly grown portions of the biofilms. Biofilm cells were lysed using a bead beater coupled with guanidium thiocyanate. Nucleic acids were extracted using phenol–chloroform and precipitated with isopropanol. The community 16S rRNA genes were polymerase chain reaction (PCR) amplified using universal eubacterial primers (27F and 1492R). Gel-purified PCR product was ligated into pCR-XL-TOPO vectors and transformed into *Escherichia coli* cells using the TopoXL kit (Invitrogen, Carlsbad, CA, USA). 16S rDNA clones were sequenced at the Center of Marine Biotechnology Bioanalytical Services Laboratory. Nearest relatives of the sequences were obtained by comparison to the

GenBank (http://www.ncbi.nlm.nih.gov/) database of rRNA gene sequences using BLAST (Altschul *et al.*, 1997). Sequence data were edited using the PreGap4 and Gap4 software applications from the Staden Package. Chimeric sequences were identified with the program Check_Chimera (http://35.8.164.52/html/index.html) of the Ribosomal Database Project (http://rdp.cme.msu.edu/index.jsp; Maidak *et al.*, 1999). Complete rRNA gene sequences were aligned to a database of >10 000 known SSU rRNA sequences with the ARB software package (Ludwig, 2004). Phylogenetic dendograms were generated with the neighbour-joining algorithm (Saitou & Nei, 1987) using *E. coli* 16s rDNA sequence positions 10–575. Branch points were confirmed using Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum parsimony (Kluge & Farris, 1969) algorithms. Bootstrap values were generated from 1000 replicate data sets with the neighbour-joining algorithm using the Phylip program (Felsenstein, 2004). Only branch points with bootstrap values greater than 50% were included in the dendogram.

RESULTS AND DISCUSSION

$CaCO_3$ distribution

Secondary electron images of biofilms cultured in the three experimental seawaters (Fig. 2) reveal that $CaCO_3$ is generally precipitated between the biofilm's microbial cells. This is particularly evident in Fig. 2C, where bacterial cells were cross-sectioned during sample preparation, thus revealing the extracellular, void-filling $CaCO_3$ matrix. The biofilms, including the control, showed no significant differences in their percentage calcification. Average weight-percentage calcification for biofilms grown in all three seawater treatments was 64 ± 4%. SEM imaging revealed that the nonbiofilm control plates contained no trace of $CaCO_3$ precipitation.

$CaCO_3$ polymorph variation with Mg/Ca_{sw}

Powder X-ray diffraction analysis of $CaCO_3$ precipitated within the biofilms revealed that the calcite:aragonite ratio increased as Mg/Ca_{sw} decreased towards the calcite stability field ($mMg/Ca_{sw} < 2$; Fig. 3A). Biofilms cultured in the modern, aragonite seawater ($mMg/Ca_{sw} = 5.2$) produced the majority (57 ± 1.0%) of their $CaCO_3$ as aragonite with lesser amounts as magnesian calcite (43 ± 1.0%; Fig. 3B). Biofilms cultured in the aragonite–calcite boundary seawater ($mMg/Ca_{sw} = 2.5$) produced the majority of their $CaCO_3$ as magnesian calcite (85 ± 1.1%) with lesser amounts as aragonite (15 ± 1.1%). And biofilms cultured in the experimental calcite seawater ($mMg/Ca_{sw} = 1.5$) produced exclusively magnesian calcite. The control biofilm exhibited relative abundances of $CaCO_3$ polymorphs (aragonite = 62 ± 1.5%; magnesian calcite = 38 ± 1.5%) that were similar to those exhibited by the biofilm cultured in the artificial aragonite seawater.

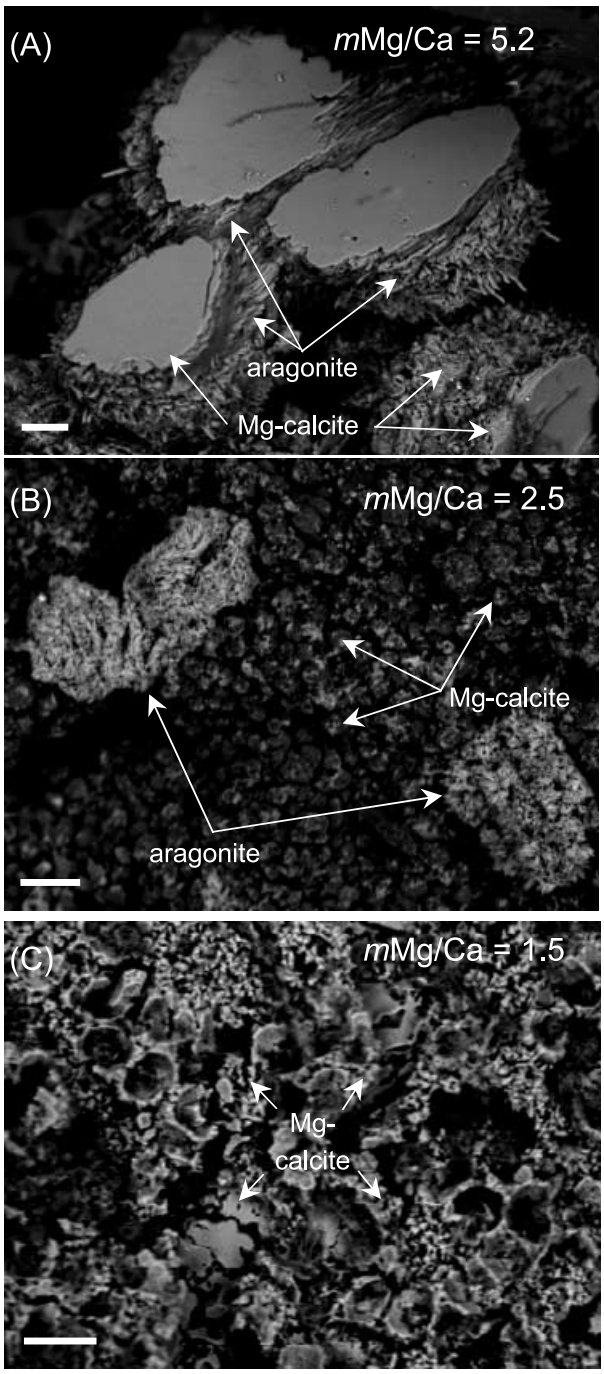

Fig. 2 Secondary electron images (JEOL 8600 Superprobe) showing distribution of aragonite (large, acicular crystals) and Mg-calcite (small, micritic crystals) precipitates within biofilms grown in the three experimental seawaters. (A) $mMg/Ca_{sw} = 5.2$; 43.0 mole-percentage calcite ($Mg/Ca_{calcite} = 0.156$); 57.0 mole-percentage aragonite. (B) $mMg/Ca_{sw} = 2.5$; 85.5 mole-percentage calcite ($Mg/Ca_{calcite} = 0.078$); 14.5 mole-percentage aragonite. (C) $mMg/Ca_{sw} = 1.5$; 100 mole-percentage calcite ($Mg/Ca_{calcite} = 0.058$). Scale bars are 20 μm. Relative abundance and spatial distribution of $CaCO_3$ polymorphs was determined by powder X-ray diffraction, SEM, and energy-dispersive spectrometry, such that elevated Sr/Ca and Mg/Ca were employed as proxies of aragonite and calcite, respectively (Ries *et al.*, 2006).

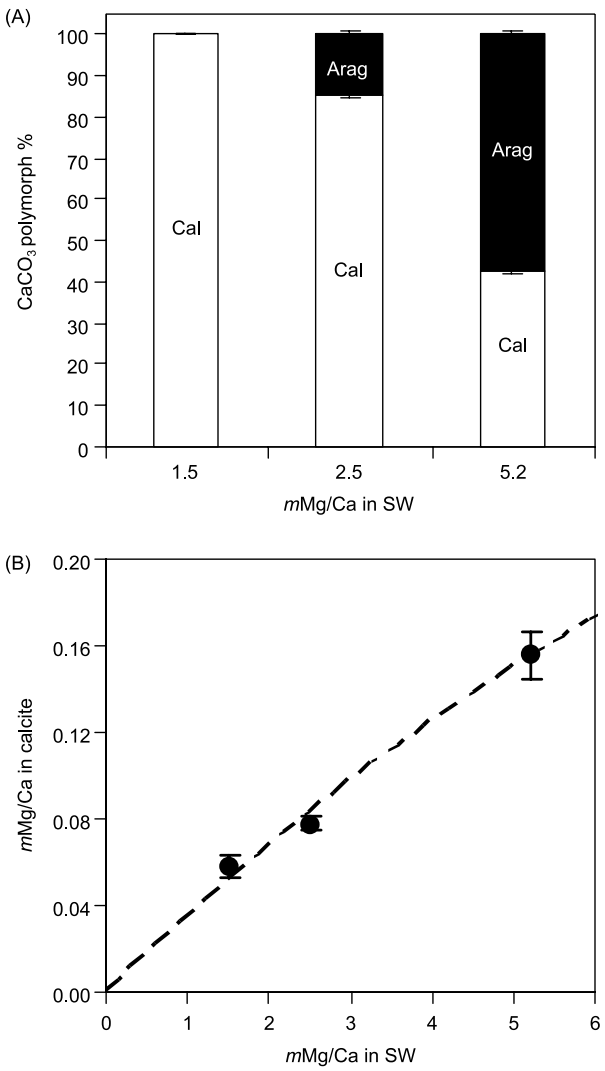

Fig. 3 Mineralogy and geochemistry of $CaCO_3$ precipitated within biofilms grown in the three experimental seawaters. (A) Relative abundance (mole-percentage) of calcite and aragonite within biofilm $CaCO_3$, determined by powder X-ray diffraction. (B) *m*Mg/Ca of calcite precipitated within biofilms, determined by powder X-ray diffraction and energy-dispersive spectrometry. Mg-fractionation algorithm (dashed curve) calculated using least squares regression is defined as $y = 0.0397x^{0.811}$ ($R^2 = 0.99$). Error bars represent instrument error and specimen variation.

Mg-fractionation in microbial calcite

The Mg/Ca ratio of the biofilm calcite ($Mg/Ca_{calcite}$) varied proportionally with Mg/Ca_{sw} (Fig. 3B). Biofilms cultured in the experimental seawaters formulated at mMg/Ca_{sw} of 5.2, 2.5, and 1.5 yielded $mMg/Ca_{calcite}$ of 0.16 ± 0.01, 0.08 ± 0.01, and 0.06 ± 0.01, respectively. Calcite within the control biofilm exhibited Mg/Ca ratios ($mMg/Ca = 0.17 \pm 0.02$) that were similar to Mg/Ca ratios of the calcite precipitated within the biofilm cultured in the artificial aragonite seawater. The relationship between Mg/Ca_{sw} and $Mg/Ca_{calcite}$ observed for the biofilms ($0.16 < Mg/Ca_{calcite} < 0.17$ in $mMg/Ca_{sw} = 5.2$ at 25 °C) generally mimics Mg-fractionation in abiotically precipitated calcite cements ($0.14 < Mg/Ca_{calcite} < 0.18$ in modern shallow seawater; Morse *et al.*, 2006) and is consistent with Mg-fractionation in calcite produced by corals (Ries *et al.*, 2006), calcareous green algae (Ries, 2005; Ries, 2006a), coralline red algae (Stanley *et al.*, 2002; Ries, 2006b), some species of coccolithophores (Stanley *et al.*, 2005), echinoids, crabs, shrimp, and calcareous serpulid worm tubes (Ries, 2004).

It should also be noted that while Mg-fractionation in biofilm calcite appears to mimic that of abiotic calcification, the observation that calcification occurred exclusively on the biofilm plates, and not at all on the nonbiofilm control plates, suggests that the calcification observed in the biofilms required biological forcing, and should thus be considered biogenic in nature.

Mechanisms of microbial calcification

Calcification within microbial biofilms results from the complex interplay of metabolic and geochemical reactions occurring within and adjacent to the biofilm matrix (Dupraz & Visscher, 2005; Altermann *et al.*, 2006). An aqueous system's affinity for calcification can be quantified by its $CaCO_3$ saturation state (Ω_{CaCO_3}):

$$\Omega_{CaCO_3} = [Ca^{2+}][CO_3^{2-}]/K_{sp},$$

such that $[Ca^{2+}]$ and $[CO_3^{2-}]$ are the molal concentrations (moles/kilogram) of Ca^{2+} and CO_3^{2-} in solution and K_{sp} is the stoichiometric solubility product of the appropriate polymorph of $CaCO_3$ [$10^{-6.37}$ and $10^{-6.19}$ for calcite and aragonite (Mucci, 1983), respectively, at 25 °C, 1 atm, and 35‰ salinity]. If $\Omega_{CaCO_3} > 1$, calcification should occur; if $\Omega_{CaCO_3} < 1$, dissolution should occur. While the saturation state of seawater with respect to Mg-calcite is also influenced by $[Mg^{2+}]$ of the precipitating solution as well as mole-percentage Mg of the Mg-calcite, adapting Ω_{CaCO_3} to incorporate the effects of Mg is problematic (see Morse *et al.*, 2006, 2007 for discussion).

The C-, O-, S-, N-, and Fe-based reduction–oxidation reactions that form the basis of microbial metabolisms greatly affect the biofilm system's PH, alkalinity, and dissolved inorganic carbon, and thus $[CO_3^{2-}]$ and Ω_{CaCO_3} of the biofilm's intercellular space, which determine whether calcification will occur (Bosak & Newman, 2005; Dupraz & Visscher, 2005; Visscher & Stolz, 2005; Baumgartner *et al.*, 2006). Simplified examples (Visscher & Stolz, 2005) of such microbial reactions that effectively *increase* $[CO_3^{2-}]$ and Ω_{CaCO_3} are:

- Photosynthesis: $CO_2 + H_2O \rightarrow CH_2O + O_2$
- Anoxygenic photoautotrophy: $HS^- + 2CO_2 + 2H_2O \rightarrow 2CH_2O + SO_4^{2-} + H^+$
- Dissimilatory iron-reduction: $CH_2O + 4FeOOH + H_2O \rightarrow HCO_3^- + 4Fe^{2+} + 7OH^-$

Table 1 Diversity of bacteria in biofilm communities determined by 16S rRNA gene sequence analysis

Phylogenetic group	Control* (*m*Mg/Ca = 5.2)	Aragonite SW (*m*Mg/Ca = 5.2)	Calcite SW (*m*Mg/Ca = 1.5)
Cyanobacteria	32%	26%	25%
α-Proteobacteria	25%	25%	23%
Bacteroidetes	15%	16%	8%
γ-Proteobacteria	10%	19%	17%
Planctomycetes	8%	6%	2%
Chloroflexi	–	6%	8%
Actinomycetes	5%	–	6%
Verrucomicrobia	–	–	4%
Legionella	–	–	4%
Acidobacteria	–	–	2%
Chlamydiales	2%	–	–
δ-Proteobacteria	2%	–	–
Gemmatimonadetes	–	1%	–
No. of clones analysed	59	68	48

*Biofilm processed shortly after removal from lagoon and not grown in experimental seawater.

- Sulphate reduction: $2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S$
- Methanogenesis: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

Examples (Visscher & Stolz, 2005) of microbial reactions that effectively *decrease* $[CO_3^{2-}]$ and Ω_{CaCO_3} are:

- Aerobic respiration: $CH_2O + O_2 \rightarrow CO_2 + H_2O$
- Sulfide oxidation: $HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$
- Ammonium oxidation: $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$
- Dissimilatory nitrate reduction: $5CH_2O + 4NO_3^- \rightarrow 5HCO_3^- + 2N_2 + 2H_2O + H^+$
- Fermentation: $3CH_2O + H_2O \rightarrow CO_2 + H_2O + C_2H_6O$

Such primary microbial metabolic reactions within the biofilm are moderated by secondary reactions associated with EPS (Reid *et al.*, 2000; Arp *et al.*, 2001; Bosak & Newman, 2005; Dupraz & Visscher, 2005). EPS is an extension of the microbial cell that maintains biofilm structure, establishes chemical microgradients by reducing rates of diffusion, may regulate intercellular processes, and functions to bind cations, elevate HCO_3^-, and nucleate $CaCO_3$ crystals (Arp *et al.*, 2001; Dupraz & Visscher, 2005).

The observation made in this study that biofilm calcification proceeds nearly abiotically with respect to polymorph distribution and Mg-fractionation (Ries, 2004) is consistent with the assertion that calcification in biofilms occurs primarily through the elevation of $[CO_3^{2-}]$ in the biofilm's intercellular space via fundamental microbial reduction–oxidation reactions that remove CO_2 and/or H^+ (McConnaughey & Whelan, 1997; Bosak & Newman, 2005; Dupraz & Visscher, 2005), rather than by elevating $[Ca^{2+}]$ via $CaCO_3$ dissolution and EPS decomposition (Dupraz & Visscher, 2005). The latter scenario of elevated Ca^{2+}, evidence of which was not observed in the present study, would decrease the Mg/Ca ratio within the calcifying fluid of the biofilm and cause its patterns of $CaCO_3$ polymorphism and Mg-fractionation (Fig. 3), with respect to ambient Mg/Ca_{sw}, to deviate from those of abiotically precipitated $CaCO_3$.

Biofilm bacterial community analysis

Clone library estimates of the microbial diversity (Table 1) and phylogenetic structure (Fig. 4) of the biofilms cultured in the artificial aragonite (*m*Mg/Ca = 5.2) and calcite (*m*Mg/Ca = 1.0) seawaters, and of the control biofilm, are highly similar. The biofilm communities from each of the seawater treatments are dominated by cyanobacteria, alpha proteobacteria, bacteroidetes, and gamma proteobacteria, which collectively comprise greater than 70% of the biofilms' bacterial community. Cyanobacteria are more abundant in the control biofilm than in either of the experimental biofilms that were subjected to artificial seawater. This may be attributable to the lower irradiance of laboratory conditions affecting growth of these phototrophs. The biofilms grown in the artificial seawaters also had greater abundances of gamma proteobacteria than bacteroidetes, while the control biofilm had a greater abundance of bacteroidetes than gamma proteobacteria. There were also slight differences in the representation of the low-abundance bacterial groups, including the planctomycetes, chloroflexi, actinomycetes, verrucomicrobia, legionella, acidobacteria, chlamydiales, delta proteobacteria, and gemmatimonadetes. Such minor differences in the relative abundances of bacterial groups among the one control and two experimental biofilms may be attributable to subtle differences between laboratory and natural conditions and/or stochastic variations in cloning or sampling efficiency.

It has recently been demonstrated that pure cultures of some sulphate-reducing bacteria specify the polymorph of the $CaCO_3$ that they precipitate due to the bacteria surface's affinity for the Mg^{2+} cation and the tendency for Mg^{2+} concentrations to be elevated adjacent to the cell as the $MgSO_4$ ion pair in solution dissociates via sulphate uptake (Van Lith *et al.*, 2003). Thus, specific microbes can potentially determine the proportions of $CaCO_3$ polymorphs precipitated within biofilms.

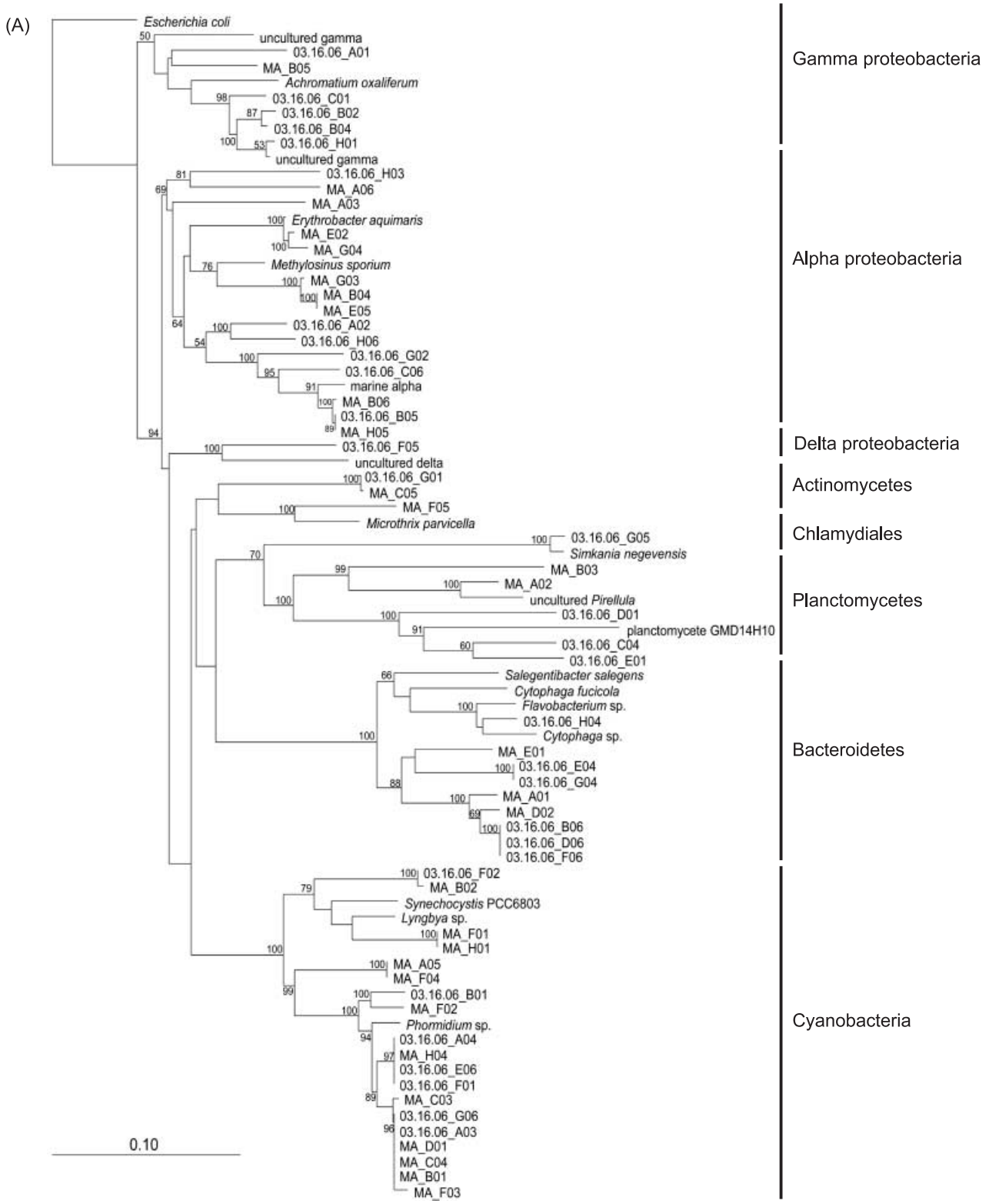

Fig. 4 Phylogenetic dendograms of clone libraries from control biofilm (A) and from biofilms grown in the artificial aragonite (B) and calcite (C) seawaters. 16S rRNA gene sequences from the present study begin with 'MA_', 'JBR_', or '03.16.06_'. Related 16S rRNA gene sequences were obtained from GenBank and were derived from cultured organisms, unless specified as uncultured. Bootstrap values are indicated at branch points. The major groups represented in the dendograms are illustrated with vertical bars to the right of the figure. Scale bars are 0.10 nucleotide changes per site.

Fig. 4 *Continued*

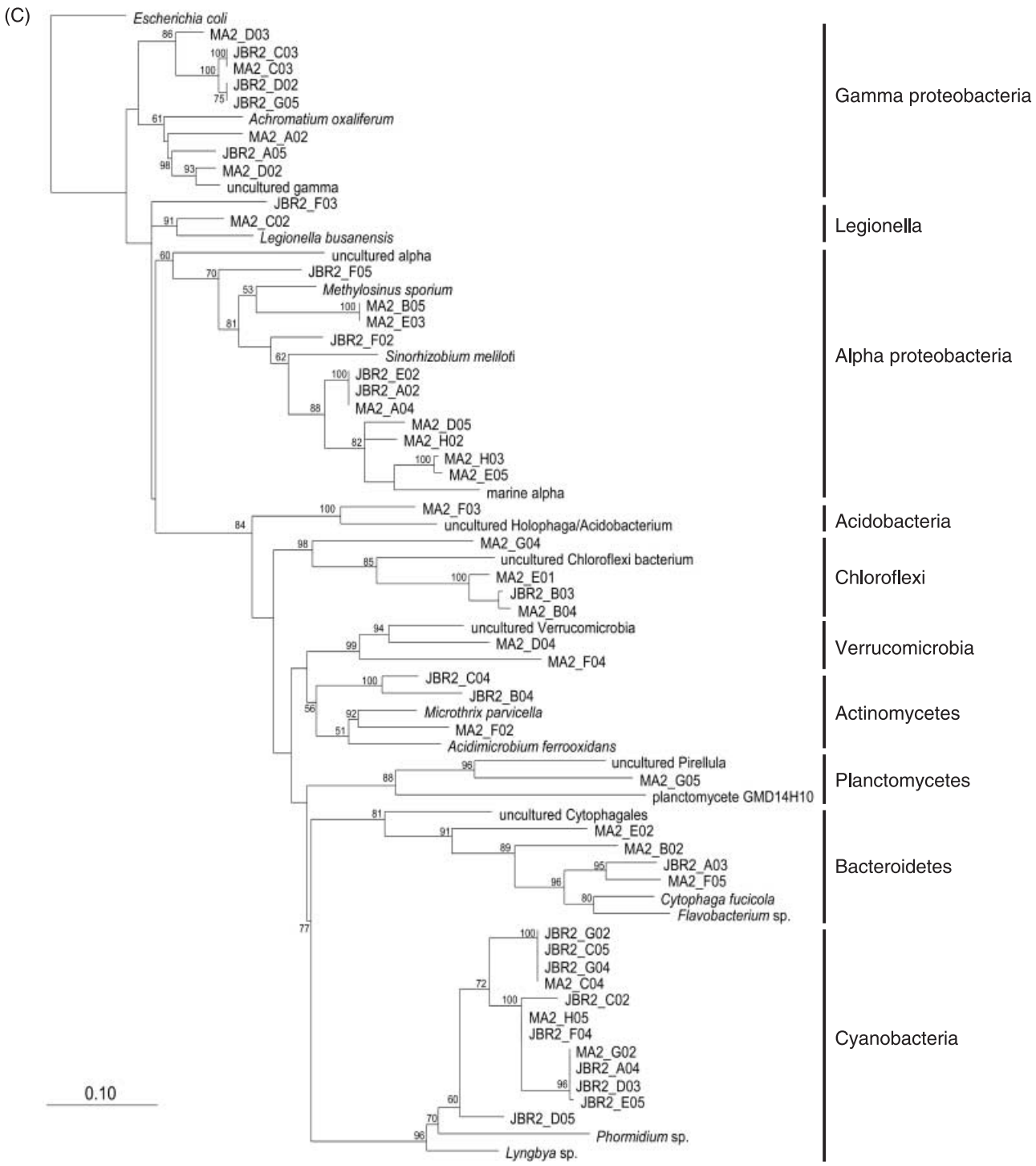

Fig. 4 *Continued*

However, it seems improbable that the minor differences in bacterial diversity and abundance that were observed between the biofilms cultured in the experimental aragonite and calcite seawaters could account for the significant differences in their $CaCO_3$ polymorph ratios. The more parsimonious explanation for the observed variation in $CaCO_3$ polymorphism and $Mg/Ca_{calcite}$ within biofilms cultured in the various seawater treatments is the prescribed difference in seawater Mg/Ca. While specific sulphate-reducing bacteria may dictate $CaCO_3$ polymorph mineralogy in pure culture (Van Lith *et al.*, 2003), the

results of the present study suggest that this ability is lost in natural, normal-salinity mixed-culture biofilms, where such polymorph-specifiers apparently constitute only a small portion of the total calcifying bacterial community.

Furthermore, the similarity of the composition and structure of the experimental biofilms to that of the control biofilm (Table 1, Fig. 4A–C), which was not subjected to the experimental seawater conditions, as well as to other natural calcifying microbial communities (Burns *et al.*, 2004; Lopez-Garcia *et al.*, 2005; Papineau *et al.*, 2005), indicates that the experimental biofilms are essentially representative of natural, calcifying microbial communities and therefore reasonable systems from which to generalize about microbial calcification. Barring phylogenetic reconstruction of fossilized DNA from lithified microbial communities, these modern, normal-salinity, calcifying biofilm systems are the next-best models for investigating the structure and function of the ancient microbial systems believed to be responsible for the construction of stromatolites, thrombolites, and microbialites throughout the geological past (Reid *et al.*, 2000).

Precambrian ocean chemistry reconstructions

The timing of aragonite and calcite sea intervals is fairly well constrained for the Phanerozoic Eon (Sandberg, 1983; Hardie, 1996; Stanley & Hardie, 1999; Lowenstein *et al.*, 2001; Horita *et al.*, 2002; Dickson, 2004). However, the distribution of calcite and aragonite seas in Precambrian time is poorly known, primarily due to the lack of indices of seawater chemistry identified in the Precambrian geological record, such as primary fluid inclusions and calcareous fossils whose skeletons can be chemically calibrated with modern representatives. Our current understanding of Precambrian Mg/Ca_{sw} is derived primarily from Hardie's (2003) hydrothermal brine–river water mixing model driven by ocean crust production rates inferred from secular oscillations in granite-pluton production in North America (Engel & Engel, 1970). Empirical evidence for secular variation in Mg/Ca_{sw} and calcite-aragonite seas in Precambrian time is limited primarily to observations of aragonite seafloor precipitates (crystal fans, early marine cements, ooids) compiled by Hardie (2003, see references therein).

Our observation that the $CaCO_3$ polymorph ratios of calcifying biofilms vary with Mg/Ca_{sw} suggests that the original mineralogy of microbial carbonates may be an archive of aragonite and calcite sea intervals throughout Precambrian time. Furthermore, the proportional relationship between biofilm $Mg/Ca_{calcite}$ and Mg/Ca_{sw} suggests that the Mg content of well-preserved, originally calcitic microbial carbonates may be a reliable monitor of Precambrian Mg/Ca_{sw} (Dickson, 2004; Ries, 2004).

An important assumption implicit in the use of the original mineralogy of microbial carbonates as a proxy for seawater Mg/Ca is that the physicochemical properties of Precambrian seawater were such that Mg/Ca_{sw} was the predominant variable influencing the polymorph mineralogy of $CaCO_3$ precipitated from seawater, as it is believed to have been throughout Phanerozoic time (Hardie, 1996; Stanley & Hardie, 1998, 1999; Lowenstein *et al.*, 2001).

Using modern soda lakes associated with volcanic regions as analogs, Kempe & Degens (1985) infer that the Earth's ocean prior to 1 Ga was a soda ocean ($HCO_3^- > Ca^{2+}$) of high alkalinity, high pH, and low Ca^{2+} and Mg^{2+} concentrations. They further argue that by 1 Ga, the gradual leaching of chlorine from the oceanic crust and the removal of dissolved carbonates via biotic and abiotic $CaCO_3$ precipitation had transformed the soda ocean into a halite ocean.

Morse & Mackenzie (1998), however, argue that if early seawater was buffered by reactions involving carbonates and silicates, then the composition of post-Hadean seawater may have been comparable to that of today. In contrast to earlier hypotheses that the Precambrian ocean was a soda ocean prior to 1 Ga (Kempe & Degens, 1985) or even 2 Ga (Grotzinger & Kasting, 1993), Morse and Mackenzie's calculations suggest that the Precambrian ocean had been a halite ocean since Late Hadean–Early Archaean time, with somewhat higher DIC and alkalinity concentrations, higher $CaCO_3$ saturation states, and possibly lower Ca^{2+} concentrations.

Grotzinger & Kasting (1993) argue that the occurrence of pseudomorphs after $CaSO_4$ minerals in the geological record back to 2 Ga suggests that the ocean was not alkaline and maintained $Ca^{2+} > HCO_3^-$ over this interval. They further propose that prior to 2 Ga, the absence of evidence for gypsum indicates either (i) such low SO_4^{2-} concentrations that $CaSO_4$ minerals were unable to precipitate, or (ii) $HCO_3^- > Ca^{2+}$, such that all Ca^{2+} was depleted during progressive evaporation of seawater via $CaCO_3$ precipitation before the gypsum field could be reached. If the latter scenario ($HCO_3^- > Ca^{2+}$) occurred, then their assertions would push the soda-to-halite ocean transformation back to 2 Ga. If the former scenario (very low SO_4^{2-}) occurred, as suggested by the Archaean record of stable isotopes of sulphur (Canfield *et al.*, 2000), then the transformation to a halite ocean could have occurred much earlier, and would not argue for a Precambrian ocean composition that was significantly different from that of today's, at least with respect to the role of Mg/Ca_{sw} in determining $CaCO_3$ polymorph mineralogy.

Hardie (2003) argues, like Morse & Mackenzie (1998), that the post-Hadean Precambrian ocean was never a soda ocean, but instead was a near-neutral halite ocean with $Ca^{2+} > HCO_3^-$, comparable to modern seawater. Hardie asserts that the extreme acidity caused by the high concentrations of dissolved HCl and CO_2 in the Earth's primordial ocean (Garrels & Mackenzie, 1971) would have fostered a global-scale acid-base titration that would have converted primordial igneous crust into aluminosilicate sediments and yielded a saline ocean with $Na^{2+} \sim Ca^{2+} > Mg^{2+} > K^+$ and near neutral pH (Garrels & Mackenzie, 1971; Lafon & Mackenzie, 1974). Hardie (2003) proposes that the elevated production of $CaCl_{2(aq)}$ relative to $CaHCO_{3(aq)}^+$, due to the predominance of HCl over CO_2

dissolved in the primordial acid rain, would have yielded $Ca^{2+} > HCO_3^-$ in this primordial ocean. Hardie cites reports of pseudomorphs after gypsum at 2.6 Ga (Simonson *et al.*, 1993) and 3.45 Ga (Lowe, 1983) in support of this line of reasoning.

Temperature, pCO_2, and $[SO_4^{2-}]$ have also been shown to influence the polymorph mineralogy of $CaCO_3$ precipitated from seawater-based solutions (Bischoff & Fyfe, 1968; Walter, 1986; Burton & Walter, 1991; Morse *et al.*, 1997). In modern seawater (S = 35 ppm, 1 atm, normal alkalinity) with *m*Mg/Ca = 5.2, the kinetically favoured $CaCO_3$ polymorph will switch from aragonite to calcite when temperature falls below 6 ± 3 °C and when pCO_2 (assuming T = 25 °C) falls between ~2600 and 3500 ppm (Morse *et al.*, 1997), which represents the range over which modern seawater will be undersaturated with respect to aragonite yet supersaturated with respect to calcite. Because the stoichiometric solubility coefficients (K_{sp}) of aragonite ($10^{-6.19}$) and calcite ($10^{-6.37}$) are relatively close, the range of calcite supersaturation states that yields simultaneous aragonite undersaturation is narrow ($1 < \Omega_{calcite} < 1.5$), and generally requires that seawater be near undersaturation with respect to calcite.

Given the ubiquity and abundance (Riding, 2000) of nearly abiotically precipitated microbial carbonates from Archaean through Neoproterozoic time, it seems improbable that the $CaCO_3$ saturation state of post-Hadean seawater was regularly constrained to such a narrow range that teetered on the edge of total $CaCO_3$ undersaturation ($1 < \Omega_{calcite} < 1.5$), a condition required by the hypothesis that elevated pCO_2 was the primary driver of calcite sea intervals (Morse *et al.*, 1997). To the contrary, it is asserted that Archaean and Proterozoic seawater actually maintained $CaCO_3$ saturation states that were much greater than modern seawater (modern seawater $\Omega_{calcite} \sim 5.7$; Kempe & Degens, 1985; Grotzinger, 1989; Grotzinger & Kasting, 1993; Morse & Mackenzie, 1998; Grotzinger & Knoll, 1999; Grotzinger & James, 2000), and thus well above the range ($1 < \Omega_{calcite} < 1.5$) that permits concomitant aragonite undersaturation and calcite supersaturation. Such assertions cast doubt over the role of pCO_2 as a primary driver of $CaCO_3$ polymorph mineralogy throughout post-Hadean time.

Sulphate has also been shown experimentally to inhibit the precipitation of both calcite and, to a lesser extent, aragonite (Bischoff & Fyfe, 1968; Walter, 1986) in seawater-based solutions. However, the effect of seawater/Mg/Ca on $CaCO_3$ polymorph specification (Leitmeier, 1910, 1915; Lippman, 1960; Müller *et al.*, 1972; Berner, 1975; Morse *et al.*, 1997; Stanley & Hardie, 1999) supersedes that of $[SO_4^{2-}]$ (Bischoff & Fyfe, 1968) when considered over the geologically realistic ranges that stable isotopes of sulphur (Canfield *et al.*, 2000; Kah *et al.*, 2004), fluid inclusions in halite (Horita *et al.*, 2002; Lowenstein *et al.*, 2003), and various ocean chemistry models (Hardie, 2003; Berner, 2004; Demicco *et al.*, 2005) suggest for Precambrian time ($0 < [SO_4^{2-}] < 20$–25 mM; $1 <$ *m*Mg/Ca < 5.2).

It is more conceivable, however, that temperature (Morse *et al.*, 1997) played a significant role in determining the primary polymorph mineralogy of microbial carbonates throughout Precambrian time. It is therefore important that such reconstructions of seawater Mg/Ca preferentially employ microbial carbonates believed to have been deposited in tropical (~25 °C) seawater. Fortunately, as $CaCO_3$ saturation states naturally increase with temperature, such warm-water carbonates are inherently more abundant than cool-water carbonates in the geological record.

It should be noted that workers have observed a transition in the mode of accretion of Precambrian microbial carbonates from one that occurs primarily via *in situ* precipitation in Archaean through Mesoproterozoic time, to one involving a combination of *in situ* calcification and the trapping and binding of loose $CaCO_3$ sediments in Neoproterozoic time and thereafter (Grotzinger & Knoll, 1999; Grotzinger & James, 2000; Sumner & Grotzinger, 2004). However, this transition in mode of accretion should have little bearing on the viability of microbial carbonates as a proxy of seawater Mg/Ca_{sw} throughout the Precambrian, as the polymorph mineralogy of abiotically precipitated $CaCO_3$ sediments that are trapped and bound in the biofilms and microbial mats and the polymorph mineralogy of $CaCO_3$ precipitated *in situ* should be comparably governed by, and thus equally indicative of, ambient seawater Mg/Ca.

The far greater challenge to using the original mineralogy of Precambrian microbial carbonates as a proxy for understanding secular variation in the major cation composition of Precambrian seawater relates to diagenesis. The aragonite polymorph is less stable than the calcite polymorph at Earth surface conditions and will thus convert to calcite over relatively short geological timescales. However, various indicators, such as trace element composition (Mg^{2+}, Sr^{2+}), quality of textural preservation, and presence of relic aragonite needles (Grotzinger & Reed, 1983; Lasemi & Sandberg, 1984, 1993, 1994; Brand, 1989), have been successfully employed to deduce the precursor mineralogy of micritic carbonates altered in this way.

Diagenesis can also cause the loss of Mg^{2+} from magnesian calcite. Yet despite the effects of diagenesis, the Mg/Ca ratio and dolomite content of well-preserved fossil echinoderms has been shown to generally track seawater Mg/Ca throughout Phanerozoic time (Dickson, 2002, 2004). Thus, the Mg/Ca ratio and dolomite content of well-preserved microbial calcite may similarly track Precambrian Mg/Ca_{sw}.

Implications for $\delta^{18}O$ and $\delta^{13}C$ isotope stratigraphy

Isotopes of carbon and oxygen are fractionated differently in calcite and aragonite due to differences in the internal vibrational frequencies of the polymorphs' carbonate ions (Rubinson & Clayton, 1969; Tarutani *et al.*, 1969). For $CaCO_3$ precipitated at 25 °C from artificial seawater, $\delta^{18}O$ and $\delta^{13}C$ are enriched in the aragonite polymorph by 0.6‰ and 1.8‰, respectively, relative to the calcite polymorph. $\delta^{18}O$ is also enriched in

high-Mg calcite (12.8 mole-percentage Mg) by 1.1‰ relative to pure calcite, indicating a 0.09‰ enrichment in $\delta^{18}O$ per mole-percentage Mg. Thus, $\delta^{18}O$ and $\delta^{13}C$ isotope records derived from Precambrian microbial carbonates (e.g. Jacobsen & Kaufman, 1999; Halverson *et al.*, 2005) may contain global, threshold excursions of up to 2‰ caused by secular variations in the primary mineralogy of the host carbonates that are independent of the biological and geological processes typically considered in the interpretation of these isotope records.

CONCLUSIONS

The effect of seawater Mg/Ca on microbial biofilm calcification was experimentally investigated to evaluate the viability of microbial carbonate mineralogy as proxy for calcite-aragonite seas throughout Precambrian time. Significantly, biofilms cultured in experimental aragonite seawater ($m\mathrm{Mg/Ca_{sw}} = 5.2$) precipitated primarily aragonite, with lesser amounts of high-Mg calcite, while biofilms cultured in experimental calcite seawater ($m\mathrm{Mg/Ca_{sw}} = 1.5$) precipitated exclusively calcite. Furthermore, the Mg/Ca ratios of the calcite precipitated within the biofilms varied proportionally with the Mg/Ca ratios of the experimental seawater. The observation that biofilm calcification mimics abiotic calcification with respect to $CaCO_3$ polymorph specification and Mg-fractionation suggests that the elevation in $CaCO_3$ saturation state leading to calcification within the biofilm occurs mainly through the elevation of $[CO_3^{2-}]$, and not through the elevation of $[Ca^{2+}]$, which would inherently change the Mg/Ca ratio of the biofilm's calcifying fluid and cause its $CaCO_3$ polymorph mineralogy to differ from that of abiotically precipitated $CaCO_3$. If, as previously asserted, the influence of seawater Mg/Ca on $CaCO_3$ polymorph mineralogy in Precambrian seawater was comparable to that in Phanerozoic seawater ($m\mathrm{Mg/Ca} \sim 2$ divided calcite and aragonite/high-Mg calcite seas, and temperature, $p\mathrm{CO_2}$, and $[SO_4^{2-}]$ played only secondary roles in $CaCO_3$ polymorph specification), then the results of the present study suggest that the primary mineralogy of microbial carbonates may be a viable proxy for calcite-aragonite seas in Precambrian time. These results invite a systematic study of the primary mineralogy and Mg content of well-preserved Precambrian microbial carbonates aimed at empirically constraining the history of Precambrian seawater Mg/Ca.

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