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Ca–Mg kutnahorite and struvite production by *Idiomarina* strains at modern seawater salinities

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Abstract

The production of Mg-rich carbonates by *Idiomarina* bacteria at modern seawater salinities has been investigated. With this objective, four strains: *Idiomarina abyssalis* (strain ATCC BAA-312), *Idiomarina baltica* (strain DSM 15154), *Idiomarina loihiensis* (strains DSM 15497 and MAH1) were used. The strain *I. loihiensis* MAH1 is a new isolate, identified in the scope of this work. The four moderately halophilic strains precipitated struvite ($NH_4MgPO_4 \cdot 6H_2O$) crystals that appear encased by small Ca–Mg kutnahorite [$CaMg(CO_3)_2$] spheres and dumbbells, which are also regularly distributed in the bacterial colonies. The proportion of Ca–Mg kutnahorite produced by the bacteria assayed ranged from 50% to 20%, and *I. abyssalis* also produced monohydrocalcite. All precipitated minerals appeared to be related to the bacterial metabolism and, consequently, can be considered biologically induced. Amino acid metabolism resulted in a release of ammonia and CO_2 that increase the pH and CO_3^{2-} concentration of the culture medium, creating an alkaline environment that favoured carbonate and struvite precipitation. This precipitation may be also related to heterogeneous nucleation on negatively charged points of biological structures. Because the nature of the organic matrix determines which ion is preferentially adsorbed and, consequently, which mineral phase is formed, the uniquely high content in odd-iso-branched fatty acids of the *Idiomarina* suggests that their particular membrane characteristics could induce Ca–Mg kutnahorite production. The Ca–Mg kutnahorite, a mineral with a dolomite-ordered structure, production at seawater salinities is noticeable. To date, such precipitation in laboratory cultures, has only been described in hypersaline conditions. It has also been the first time that biomineralization processes have been related to *Idiomarina* bacteria.

Keywords: Biomineralization; Mg-rich carbonates; Mg-phosphates; γ-*Proteobacteria*

1. Introduction

Carbonate precipitation has been widely discussed for the last few decades with regard to microbial processes. Such processes have played an important role mediating carbonate production throughout the Earth's history (e.g., Knorre and Krumbein, 2000; Wright and Wacey, 2004, 2005; Dupraz and Visscher, 2005; Wright and Oren, 2005; Altermann et al., 2006). In particular, precipitation of microbial dolomite has been the focus of extensive research and special attention has been paid to so-called "dolomite problem" (e.g., Vasconcelos et al., 1995; Burns et al., 2000; Wright and Wacey, 2004, 2005). While dolomite is relatively rare in modern natural environments, the abundance of dolomite in ancient sediments suggests that the process responsible for its precipitation in the past may have been significantly different, or operated at much larger scale.

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In fact, this mineral is of key importance for understanding biogeochemical processes and cycles in past and modern environments, linking the geosphere with the biosphere (e.g., Burns et al., 2000; Wright and Wacey, 2005; Vasconcelos et al., 2006). Microbial dolomite precipitation has been related to bacterial sulphate reduction and methanogenesis (e.g., Van Lith et al., 2003; Wright and Wacey, 2004, 2005; Wright and Oren, 2005; Vasconcelos et al., 1995, 2006; Altermann et al., 2006), and more recently aerobic respiration has also been demonstrated to induce dolomite production at the water–sediment interface (Sánchez-Román, 2007). An understanding of the microbiogeochemical processes involved in the bacterially mediated precipitation of dolomite may offer insight into how other Mg-rich carbonates form in similar environments. In this sense, recent experiments have revealed that Ca–Mg kutnahorite, a mineral with a dolomite-ordered structure, can also be precipitated by bacteria (Rivadeneyra et al., 2006). Kutnahorite, $CaMn(CO_3)_2$, is a calcium manganese carbonate mineral (Peacor et al., 1987) member of the dolomite group. However, Ca-rich and Mg-rich phases with the kutnahorite structure have been described, suggesting a solvus in the Ca and Mg-rich and the Mn-rich members (Goldsmith, 1983; Mucci, 2004). As has been the case for dolomite, precipitation of Ca–Mg kutnahorite crystals have been obtained in hypersaline bacterial culture experiments (Rivadeneyra et al., 2006). Thus, available data suggest that salinity is an important factor controlling microbial Mg-rich carbonate production. In this context, this work was designed to investigate the bacterial precipitation of Mg-rich carbonates at modern seawater salinities. With this objective, marine bacteria were isolated in order to check their biomineralization capability. One of the isolated strains precipitated Mg-rich carbonate. This bacterium was identified as a new strain of the *I. loihiensis* species. Due to the widespread distribution of *Idiomarina* genus in marine and hypersaline habitats (Martínez-Cánovas et al., 2004) different strains were investigated. Since 2000, when the *Idiomarina* genus was proposed by Ivanova et al. (2000), numerous species of *Idiomarina* have been isolated (i.e., *I. abyssalis* and *I. zobelli* from the Pacific Ocean deep-sea, Ivanova et al., 2000; *I. loihiensis* from hydrothermal vents at a depth of 1296 m, Donachie et al., 2003; *I. baltica* from surface water of the central Baltic Sea, Brettar et al., 2003; *Idiomarina seosinensis* from hypersaline water of a solar saltern in Korea, Choi and Cho, 2005; *Idiomarina fontilapidosi* and *Idiomarina ramblicola* from a hypersaline wetland and a hypersaline rambla, respectively, Martínez-Cánovas et al., 2004; *Idiomarina homiensis* from seashore sand in Korea, Kwon et al., 2006) Members of *Idiomarina* share many phenotypic characteristics with other heterotrophic, oxidative, marine and halophilic members of the γ-*Proteobacteria*. Nevertheless, one eminent feature of the genus *Idiomarina* is its high content of iso-branched fatty acids, which is atypical of *Proteobacteria* with the sole exception of the *Xanthomonas* branch (Martínez-Cánovas et al., 2004). They can also be distinguished from other marine bacteria by their physiological characteristics, in particular their ability to grow in a broad range of temperatures, pH values, and NaCl concentrations (Martínez-Cánovas et al., 2004) Within the scope of this investigation the biomineralization capability of the isolated novel strain from surface seawater (the MAH1 strain) and three more related strains from culture collections were used for carbonate production experiments.

2. Experimental procedures

2.1. Bacterial strains

For this study, four bacterial strains belonging to the genus *Idiomarina* were used. One was isolated from a surface seawater sample (westernmost Mediterranean) within the framework of this research (strain MAH1) while the others three were obtained from bacterial-type culture collections: *I. abyssalis* (American Type Culture Collection, ATCC BAA-312), isolated from a depth of 4000–5000 m in the north-western area of the Pacific Ocean (Ivanova et al., 2000); *I. loihiensis* (Deutche Sammlung von Mikroorganismen, DSM 15497), isolated from hydrothermal vents at a depth of 1296 m on the Loihi Seamount, Hawaii (Donachie et al., 2003) and *I. baltica* (DSM 15154), isolated from surface water of the central Baltic Sea (Brettar et al., 2003).

2.2. Culture media

For biomineralization experiments, the solid medium used was the marine medium (MM) (% w/v, yeast extract 0.5%, triptone 1%, purified agar–agar 2% in seawater from the westernmost Mediterranean Sea, pH 7.6. Seawater was previously filtered with a 0.2 μm porous membrane to eliminate particulate material). For the novel strain identification, marine agar (MA) and marine broth (MB) media (DIFCO), as well as tryptic soy agar (TSA; BBL) were used. The MB medium was also used for liquid cultures and for preparing inocula. The solid media (MM and MA) were nutritive solutions solidified by agar–agar. These types of solutions, extensively used for microbial cultivation, have a structure that supports bacterial growth while allowing the nutrients and bacterial metabolites (CO_2, NH_3, etc.) to diffuse at a slow rate.

All liquid and solid media were sterilized by autoclaving for 20 min at 120 °C. The liquid medium (MB) was placed in 20 ml tubes with 5 ml per tube and the solid ones in Petri dishes at 20 ml per dish.

2.3. Cell-sample preparation for electronic microscopy

A 48-h-old culture in liquid medium MB was used. For transmission electron microscopy (TEM, Zeiss 10C), negative staining of MAH1 bacterium with uranyle acetate (2% for 1 min at room temperature) was prepared. For scanning electron microscopy (SEM, LEO Carl Zeiss GEMINI-1530),

cell samples were bound to poly-L-Lys-coated slides (at 4 °C) and fixed in glutaraldehyde 4% in 0.2 M cacodylate buffer with 0.4 M sucrose and 0.1% NaCl (in order to reach an osmolality of 1205 mOsm similar to the MB medium). After this, postfixing with osmium tetroxide (1% in the same buffer) for 1 h was performed. Following dehydration at room temperature, the samples were critical-point CO_2 dried (Polaron, CPD5501 Critical Point Drier) and coated with carbon.

2.4. Isolation and characterization of the novel bacterium strain MAH1

Bacterial strain MAH1 was isolated from a surface water sample of the westernmost Mediterranean Sea. The MAH1 strain was deposited in the Spanish Type Culture Collection (CECT) (http://www.cect.org), under reference CECT 5996. Characterization of this strain was performed by Polyphasic taxonomy that takes into account all available phenotypic and genotypic data and integrates them in a consensus type of classification, framed in a general phylogeny derived from 16S rDNA sequence analysis.

A phenotypic characterization was made and physiological and biochemical features were established according to the methods described by Mata et al. (2002) but using MA and MB media. Bacterial tolerance or requirement of NaCl was assayed on TSA with 0.5–23% (w/v) NaCl at 30 °C for 10 days. The temperature range for growth was tested on MA plates incubated at 2–45 °C. The Biolog GN system (Biolog, Inc.) and the API ZYM system (bioMérieux Vitek) were used to determine the biochemical profile, following the instructions of the manufacturer. Cell morphology was visualized with TEM and SEM, from samples of the 48-h-old liquid cultures in MB medium. Chemotaxonomic analyses (DNA–DNA hybridization, DNA base composition in G + C and polar lipids pattern) were performed in the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) Braunschweig, Germany. The complete sequence of the 16S rDNA (1447 pb) was obtained in the Institute of Radiochemistry, Forschungszentrum Rossendorf, Germany. Phylogenetic analyses were performed using MEGA 3 (Kumar et al., 2004), after multiple alignments of the data using CLUSTAL_X (Thompson et al., 1997).

2.5. Mineral production and characterization

The four strains studied were cultivated in MB medium for 48 h at 30 °C in order to prepare inocula. Aliquots of 25 µl were streaked on the MM medium and incubated at 20 °C for 30 days. As control, plates of MM medium non-inoculated and inoculated with aliquots of 25 µl of the some bacterial cultures but autoclaved, were incubated in parallel. All plates were examined once daily with the naked eye and with optical microscopy (at a total magnification of 100) to detect any possible precipitates formed. The pH was measured at the end of the experiment using pH-indicator paper (Merck) applied directly on the culture medium surface. To recover the precipitate formed, a needle was used for the largest crystals, and the small ones were recovered by melting the solid medium in a microwave oven (600 W for 50 s). After this, crystals were washed with distilled water to eliminate culture medium remains and cell debris. These experiments were replicated three times.

Precipitate composition and morphologies were studied by SEM (LEO Carl Zeiss GEMINI-1530), coupled with energy-dispersive X-ray (EDX) microanalysis (QX, 2000 Link, at 20 kV) on carbon-coated samples. Precipitate mineralogy was determined by X-ray diffraction (XRD) using a Bruker D8 Advance diffractometer. Scans were run from 3° to 80° 2θ and an internal standard (metallic silicon) was added to selected samples for a precise mineral identification. Diffractogram interpretations were made using X-powder software (http://www.xpowder.com, Martín-Ramos, 2004). This software uses least-square methods to refine the unit-cell parameters of crystalline phases to determine the exact term of any isomorphic mineral series.

3. Results

3.1. MAH1 bacterial strain characterization

This novel strain was found to be Gram-negative, chemo-organotrophic, and aerobic. It requires NaCl (0.7–20%, optimum 2%, thus it is a moderately halophilic or halotolerant bacterium) and grows over a range of temperatures (2–43 °C, optimum between 28 and 37 °C). Colonies of MAH1 after incubation for 48 h on MM and MA were translucent beige to yellow, 2 mm in diameter, circular, low convex, smooth, shiny, entire and mucoid. When it was cultivated in the liquid medium MB for at least one week, an abundant production of an extracellular polymeric substance (EPS) was detected (the other three bacteria used in this work also produced EPS and more or less mucoid colonies in MB and MM media, respectively).

Electron microscopy revealed that cells had a slightly curved rod shape (0.3–0.5 µm in diameter and 0.6–2 µm in long; Fig. 1A–C) and that they were motile by a single polar flagellum (Fig. 1A). They were visible either singly or in pairs, often forming cell aggregates (Fig. 1A–C).

The strain MAH1 displayed a DNA base composition in G + C of the 48.2 mol%. The similarity of the 16S rDNA sequence and of the DNA–DNA hybridization with respect to *I. loihiensis* (DSM 15497) was 100% and 96.1%, respectively. Polar lipids were predominantly odd-numbered and iso-branched (15 and 17). All these results clearly indicate that this strain belongs to the species *I. loihiensis* (Donachie et al., 2003). The main differences between the species type (DSM 15497) and MAH1 strains are given in Table 1, which supports the description of MAH1 as a novel strain. The phylogenetic tree resulting from neighbour-joining is shown in Fig. 2.

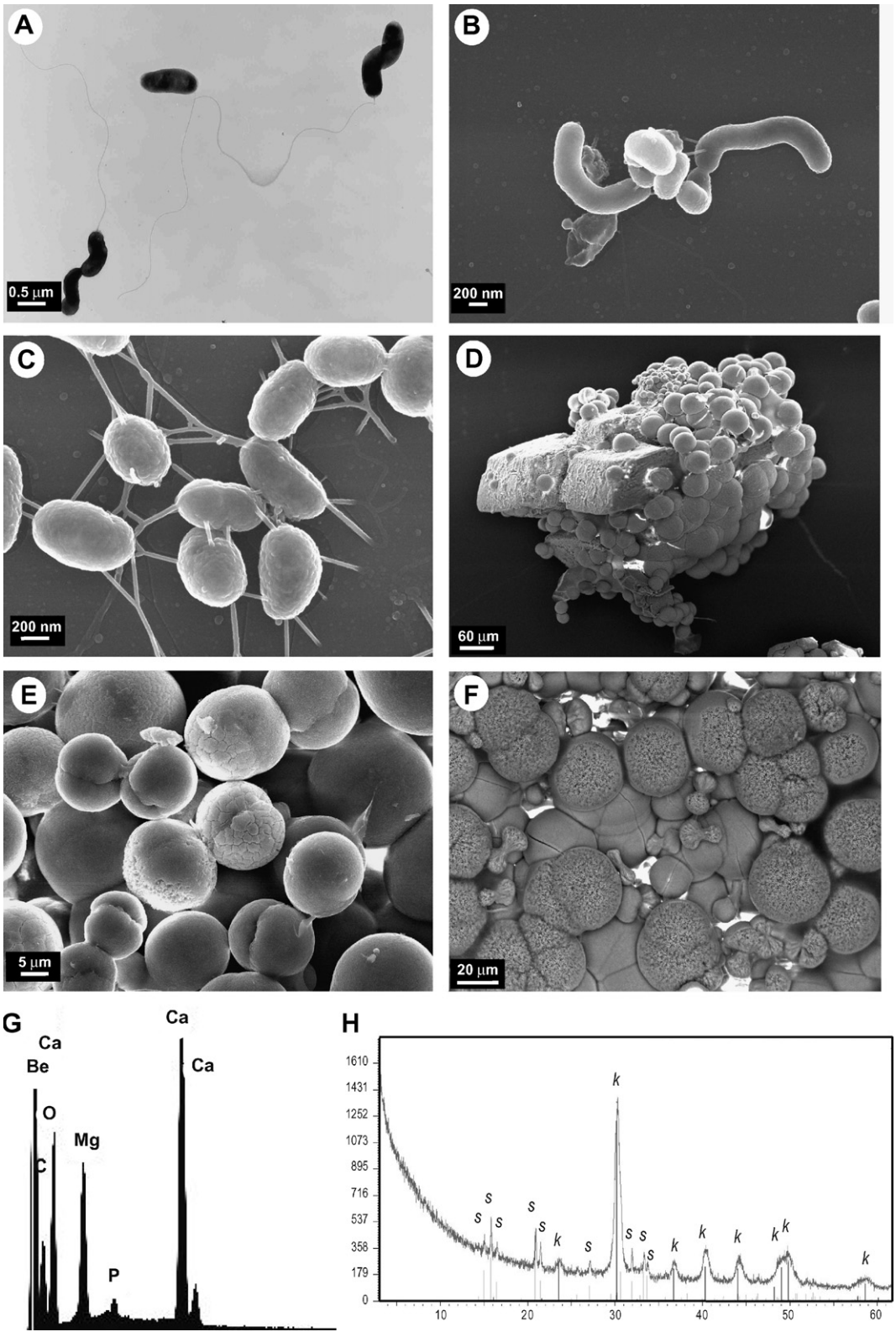

Fig. 1. Bacterial cells of *Idiomarina loihiensis* MAH1 and crystals produced: (A) TEM micrograph of negatively stained cells. (B and C) SEM micrographs of carbon-coated cells. (D) Struvite crystal encased by kutnahorite spherules. (E) SEM micrograph showing morphologies and texture of kutnahorite. (F) Backscattered image of kutnahorite spheres and dumbbells also showing internal textures. (G) SEM–EDX microanalysis of kutnahorite crystals. (H) Representative X-ray diffractogram (intensity vs. 2θ) of precipitates produced by *I. loihiensis* DSM 15497; s and k: struvite and Ca–Mg kutnahorite peaks, respectively.

Table 1
Selected phenotypic characteristics for the differentiation of *Idiomarina loihiensis* MAH1 strain nov. from type strain *I. loihiensis* DSM 15497

Characteristic	*I. loihiensis* MAH1	*I. loihiensis*[a] DSM 15497
G + C content (mol%)	48.2	47.4
NaCl range (% w/v)	0.7–20	0.5–20
NaCl optimum (% w/v)	2–6	7.5–10
Temperature range (°C)	2–43	4–46
Biolog GN2		
Glycogen	−	+
Acetic acid	±	+
cis-Aconitic acid	−	+
Citric acid	−	+
D-L-Lactic acid	−	+
Malonic acid	−	+
Succinic acid	±	−
L-Alanine	±	+
L-Alanyl-glycine	±	−
Glycerol	−	+
L-Alaninamide	+	−
L-Ornithine	±	−
Fatty acid		
$C_{11:0}$ iso	1.7	2
$C_{13:0}$ iso	1.5	1.8
$C_{15:0}$ iso	29	32.6
$C_{17:0}$ iso	12.6	11
Habitat	Westernmost Mediterranean Sea (surface)	Hydrothermal vent, Hawaii

[a] Data from Donachie et al. (2003).

3.2. Mineral precipitate production

Light microscopy (at a total magnification of 100) revealed that the investigated strains precipitated some crystals in the bacterial colonies after 3–4 days of incubation with typical polyhedral or pseudo-polyhedral struvite ($NH_4MgPO_4 \cdot 6H_2O$) morphology (Fig. 1D). Following 7–10 days of culture growth, small spheres and dumbbells were also detectable, these gradually covering the struvite crystals and increasing in abundance in the bacterial colonies. After two weeks the crystal precipitates were visible with the naked eye. These precipitates were not detected in any of the controls. The pH values of the plates inoculated with the *Idiomarina* bacteria after 30 days of the incubation was 9.0–9.3 while it was constant (7.6) in the controls.

Crystal composition and morphology were determined by EDX microanalytical data and SEM observations, respectively. These observations revealed that struvite crystals were encased by small spheres and dumbbells of Mg-rich carbonate (Fig. 1D and G), which were also regularly distributed throughout the bacterial colonies (Fig. 1E and F). Carbonate spheres and dumbbells appeared to start crystallizing as porous textures that evolved into smooth surfaces (Fig. 1E and F). Dumbbells seemed to be initial morphologies that would evolve into perfect spheres (Fig. 1E and F).

X-ray diffraction was performed for crystalline phase identification. This was done using X-powder software (Martín-Ramos, 2004) and refining the unit-cell parameters. *I. abyssalis* precipitated struvite, monohydrocalcite and Ca–Mg kutnahorite in proportions around 45%, 15% and 40%, respectively. *I. baltica* precipitated Ca–Mg kutnahorite as the most abundant mineral phase, 75%, with lesser proportions of struvite, 25%. For *I. loihiensis,* struvite and Ca–Mg kutnahorite were also the minerals precipitated at a proportion of around 50% each. The novel strain MAH1 precipitated abundant struvite, up to 80% and lesser proportions of Ca–Mg kutnahorite. Despite the different proportions found in the cultures, all the strains precipitated Ca–Mg kutnahorite (Fig. 1H).

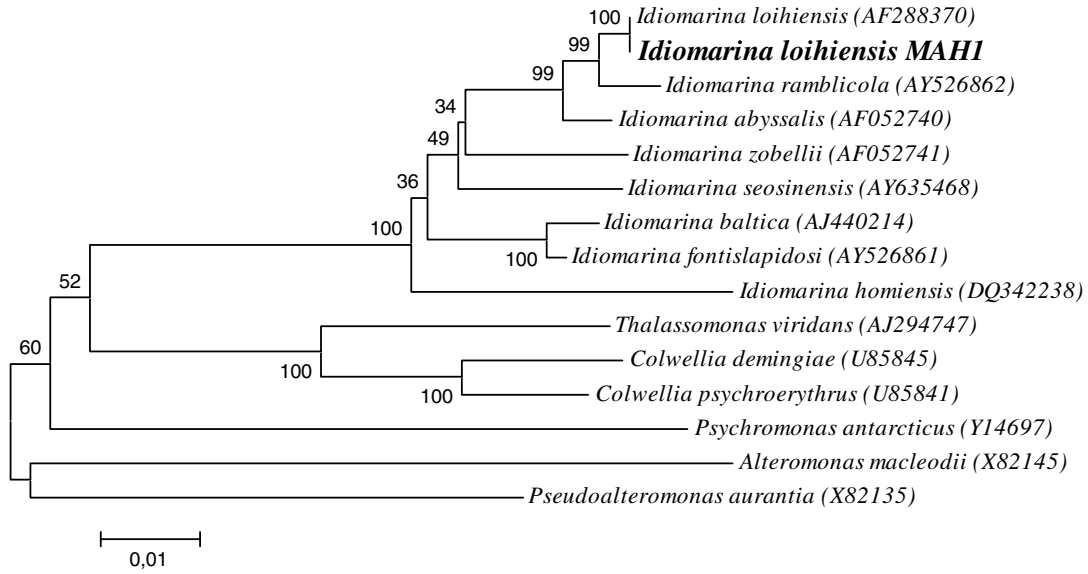

Fig. 2. Phylogenetic tree deriving from a neighbour-joining analysis of the 16S rDNA gene sequences of the *Idiomarina* species and other species belonging to related genera of γ-*Proteobacteria*. Bar, 0.01 substitutions per nucleotide position.

4. Discussion

As the scope of this work was to investigate the Mg-rich carbonate production by marine bacteria at seawater salinity, seawater samples were used to isolate marine bacteria. In order to explore the biomineralization potential of the marine strains at such salinity, the MM medium was prepared. Among the numerous bacteria growing in this medium, the strain MAH1 was selected and further identified because of its Mg-rich carbonate production capability. Since this novel strain was identified as *I. loihiensis*, other *Idiomarina* strains from bacterial culture collections were also cultivated in the same medium to assay the Mg-rich carbonate production. These strains, as indicated in Section 2.1, are representatives from diverse marine habitats.

4.1. Mineral precipitation by Idiomarina strains

According to our results, the four strains assayed produced Ca–Mg kutnahorite and struvite at seawater salinity conditions. All precipitated minerals appeared to be related to the microbial growth and were observed within the bacterial colonies. Consequently, production of these minerals can be considered biologically induced as defined by Lowenstam and Weiner (1989). Bacteria play both an active and a passive role in mineral precipitation by increasing alkalinity in the microenvironments and by providing abundant reactive sites in the EPS, cell walls and external sheaths of bacterial cells that bind dissolved mineral-forming elements. Crystals may then nucleate and grow from an oversaturated solution on the outside surface of individual cells (Wright and Oren, 2005 and references herein). The EPS matrix represents an extension for the microbial cell and functions as a chelator for cations and it can also be the template for crystal nucleation (Dupraz and Visscher, 2005).

Regarding bacterially induced carbonate precipitation, it may occur as a consequence of the bacterial presence and as a by-product of microbial metabolic processes (e.g., Knorre and Krumbein, 2000; Ben Chekroun et al., 2004). Among others, two factors, that are controlled by microbial processes and physicochemical characteristics, govern precipitation: saturation index, determined by the pH, $[Ca^{2+}]$ and $[CO_3^{2-}]$, and the exopolymeric substances (Dupraz and Visscher, 2005). Subsequently, many types of bacteria may thus enable such precipitation when growing in adequate media (Boquet et al., 1973). In the particular case of the *Idiomarina* strains cultivated in MM medium, with amino acids and peptides (as source of carbon, nitrogen, and energy), amino acid metabolism resulted in a release of ammonia and CO_2, increasing pH and, therefore, $CO^{2-}_{3(aq)}$ concentration. When a sufficient supersaturation with respect to a particular calcium carbonate phase is reached, the precipitation of such a phase is induced. On the other hand, it has also been suggested that specific attributes of certain bacteria promote calcium carbonate formation (Hammes et al., 2003 and references herein). Precipitation of carbonates may thus be related to heterogeneous nucleation on negatively charged bacterial superficial structures. These functional groups are able to attract positive ions such as Ca^{2+}, as *in vitro* studies have shown (e.g., Beveridge et al., 1983; Thompson and Ferris, 1990). The "organic matrix-mediated biomineralization" model attempts to explain this type of nucleation (Mann, 2001). The nature of the organic matrix determines which ion is preferentially adsorbed and, consequently, which mineral phase is formed. Bacterial carbonate precipitation could therefore be strain specific. For instance, bacteria that preferentially adsorb Mg^{2+} onto their membranes induce dolomite formation, whereas calcite precipitation is induced by preferential adsorption of Ca^{2+} (Van Lith et al., 2003). In this sense, a significant characteristic of the *Idiomarina* genus is its unique high content in odd-iso-branched fatty acids, suggesting that this particular membrane characteristic would induce Ca–Mg kutnahorite production. In fact, this production is not related to the medium composition since other bacteria also cultivated in the MM medium did not produce this mineral. On the other hand, the role of the EPS in the carbonate precipitation and resulting morphologies has been demonstrated by several authors (e.g., Dupraz and Visscher, 2005; Braissant et al., 2007; Ercole et al., 2007). Subsequently, a potential EPS influence in kutnahorite production can be suggested.

Regarding sphere and dumbbell morphologies of Ca–Mg kutnahorite and monohydrocalcite precipitates, these are common to other biogenic carbonates (e.g., González-Muñoz et al., 2000; Braissant et al., 2003; Sánchez-Román et al., 2007). Habit and morphologies of bacterial precipitates are influenced by viscosity of medium (Buczynski and Chafetz, 1991). In fact, in a gellified culture medium that contains calcium and magnesium, the counter diffusion of microbial metabolites and cations contained in the culture medium produces spatial-temporal gradients of concentration that create supersaturation conditions with significant implications in the development of the morphology of the precipitates (González-Muñoz et al., 2000). Buczynski and Chafetz (1991) proposed that carbonate spherules are the final stage of dumbbell growth, and Ben Chekroun et al. (2004) also demonstrated that vaterite spheres are the final growth stage of dumbbell fibrous-radiated aggregates.

Concerning the phosphate phase also originated in the cultures, struvite, it rarely occurs in nature but is a common product of bacterial precipitation. Robinson (1889) was the first to report the bacterial production of struvite that could be the consequence of the combination of ammonium ions produced by the metabolism of nitrogenous compound with phosphate and magnesium present in the environment. In a recent study, Sánchez-Román et al. (2007) have investigated the struvite production by moderately halophilic bacteria, and reported that these bacteria, belonging to 19 species from three genera, precipitated both struvite and carbonate minerals. They suggest that the moderately halophilic bacteria are more adapted

for the struvite precipitation than other nonhalophilic ones. In this sense, struvite precipitation by *Idiomarina* strains confirms that seawater salinities are also suitable for struvite bacterial production, which is of special interest for investigation of conditions leading to struvite formation. Although further work is required concerning this point, obtained results suggest that bacterial metabolism provide the necessary NH_3 and CO_2 required for supersaturation and crystal precipitation. At later stages of bacterial growth, saturation index for carbonate precipitation is reached. Initial precipitation of struvite may be related to the inhibitory effect of PO_4^{3-} on the calcite precipitation (Rivadeneyra et al., 1985). Thus, consumption of this ion will contribute to carbonate precipitation following that of struvite.

4.2. Natural environments and implications of kutnahorite production

As the obtained Ca–Mg kutnahorite is a mineral with a dolomite-type ordered structure, its precipitation at seawater salinity adds interest concerning the "dolomite problem". This has been a controversial issue for over a century since it revealed to be of capital importance in past and present biogeochemical processes (e.g., Vasconcelos et al., 1995; Burns et al., 2000; Van Lith et al., 2003; Wright and Wacey, 2004, 2005; Wright and Oren, 2005; Altermann et al., 2006). It has been demonstrated that sulphate-reducing bacteria promote dolomite precipitation under anoxic conditions (e.g., Van Lith et al., 2003; Vasconcelos et al., 1995, 2006), and also that aerobic respiration has been related to dolomite production at the water–sediment interface (Sánchez-Román, 2007). However, most of the Mg-rich carbonate (dolomite, Mg-calcite, Ca–Mg kutnahorite) precipitation experiments to date have dealt with hypersaline environments. In this regard, the precipitation of Ca–Mg kutnahorite by the investigated *Idiomarina* strains strengthens the hypothesis that the precipitation of a carbonate with a dolomite-type-ordered structure may also occur in marine environments at standard salinity. Precipitation of "ordinary" kutnahorite (a Mn-rich carbonate) is unlikely due to Mn concentration in seawater. However, further investigation with Mn-rich media could shed light on bacterial precipitation of this mineral phase. All the results found open an exciting field in which carbonate production in natural environments can be further explored and better understood.

Ca–Mg kutnahorite precipitation within laboratory cultures has been previously reported by Rivadeneyra et al. (2006) using *Chromohalobacter marismortui*. As mentioned for experiments in Mg-rich carbonate production, mineral production and optimum bacterial growth occurred at high salinities. Although excess salt is relatively common in natural environments, seawater is the most widespread natural environment and it is therefore of extreme interest to demonstrate that there bacteria can induce Mg-rich carbonate precipitation. Many more bacterial groups and respective metabolism processes should be investigated in order to achieve a complete view of microbial carbonate precipitation in natural environments.

5. Conclusions

In the exploration of microbial Mg-rich carbonate production at modern seawater salinities, it has been demonstrated that four investigated *Idiomarina* strains have biomineralization capability, in particular that to precipitate Ca–Mg kutnahorite, which is especially significant since it has a dolomite-type-ordered structure. Precipitation of this mineral within laboratory cultures has been previously reported under hypersaline conditions. However, since seawater is the most widespread natural environment, our results suggest that these conditions may also be a key scenario for microbial precipitation of Mg-rich carbonates. This opens a fascinating field for further exploration of different bacterial groups regarding microbial Mg-rich carbonate production in present and past marine environments. Furthermore, our results suggest that the abundant EPS production by the studied *Idiomarina* strains, in particular in the liquid medium, could promote the colonization of proteinaceous particles in their natural environments. These colonies and microniches could result in local conditions for carbonate precipitation.

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