Characterization of the Extracellular Polymeric Substances (EPS) of *Virgibacillus* strains capable of mediating the formation of high Mg-calcite and protodolomite

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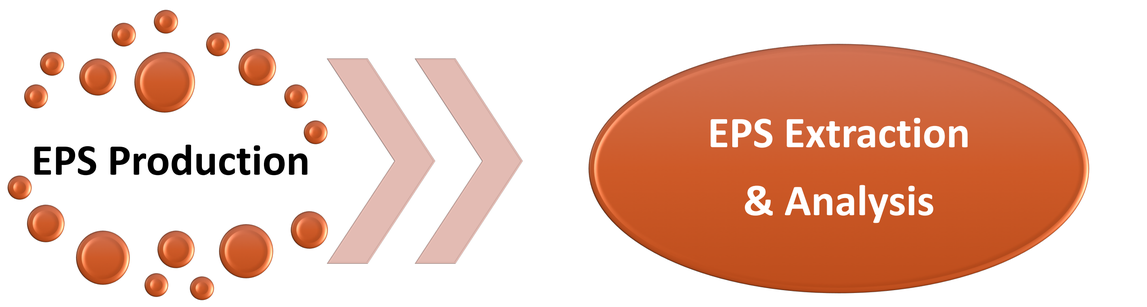
Abstract

The origin of dolomite –a common carbonate mineral– is the subject of an ongoing debate. It has been proposed that extracellular polymeric substances (EPS) excreted by microbes contain specific organic molecules that facilitate the incorporation of magnesium in the carbonate minerals. We characterized the EPS produced under different conditions by measuring total carbohydrate (TCHO), total protein (TP) content and by (FTIR) analysis. We report the formation of Mg-carbonates with a mol% Mg higher than 41% exclusively in association with EPS rich in carbohydrates (TCHO > than 75% of the total EPS mass). Moreover, FTIR analysis of these EPS revealed the presence of protein secondary structures (e.g., β-sheets) known to favor mineral nucleation. These results suggest that some organic molecules with specific functional groups (e.g., carboxyl and hydroxyl groups) may be of key importance for overcoming the kinetic barriers that else prevent the incorporation of Mg into carbonate minerals, a crucial step for the formation of dolomite in natural environments.

Introduction

Dolomite is an important Mg-carbonate mineral characterizing many oil and gas reservoirs. However, the origin of dolomite and the mode of its formation remain poorly constrained. The microbial model for dolomite formation is one of the various proposed hypothesis/solutions to solve the dolomite problem. The activities of numerous microorganisms including bacteria can overcome the kinetic energy barrier required for dolomite formation. The exact mechanism(s) of biomineralization are still controversial. EPS are mainly composed of polysaccharides and proteins (Decho & Gutierrez, 2017). In the evaporitic environments in Qatar Sabkhas, a high diversity of the bacterium *Virgibacillus* was evidenced in association with diversity of minerals (Al Disi et al., 2017). it is proposed that high temperature and subsequent increase in salinity and supersaturation caused by the strong evaporation may promote an ecological stress that stimulates the extensive synthesis of EPS by microorganisms (Bontognali et al., 2010). EPS of three bacterial strains previously isolated form Dohat Faishakh in Qatar were used for studying the EPS production. Different ranges of temperature and salinity were applied to modulate the EPS synthesis using an appropriate Mg^{+2}:Ca^{+2} ratio of 6. Two mineral forming *Virgibacillus* strains (DF112 and DF2141) were selected to exhibit several differences in their mineral forming profiles. One non-mineral forming *Bacillus licheniformis* strain (DF141) was selected as producer of EPS not appropriate for mineral formation.

Methods



Results

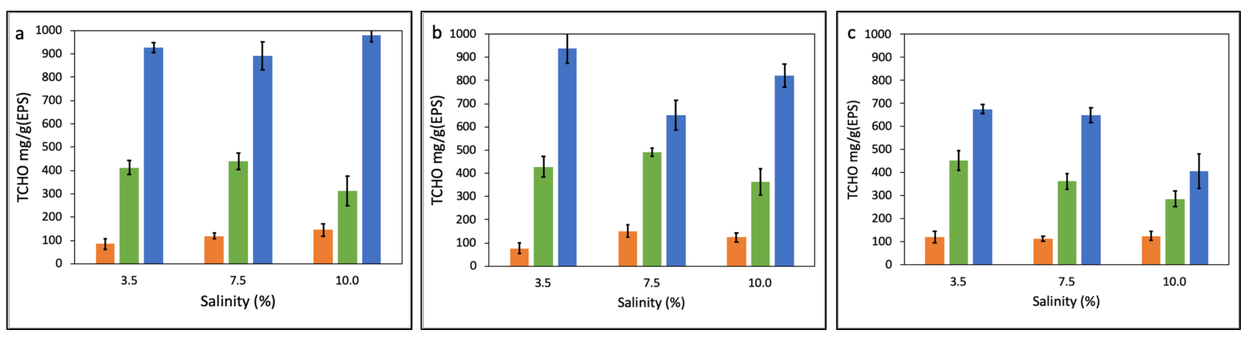


Figure 1: Total Carbohydrate of the EPS produced by mineral forming strains: a) DF112, b) DF2141 and non-mineral forming strain c) DF141 at different salinities and incubation temperatures of 20 ◦C, 30 ◦C and 40 ◦C.

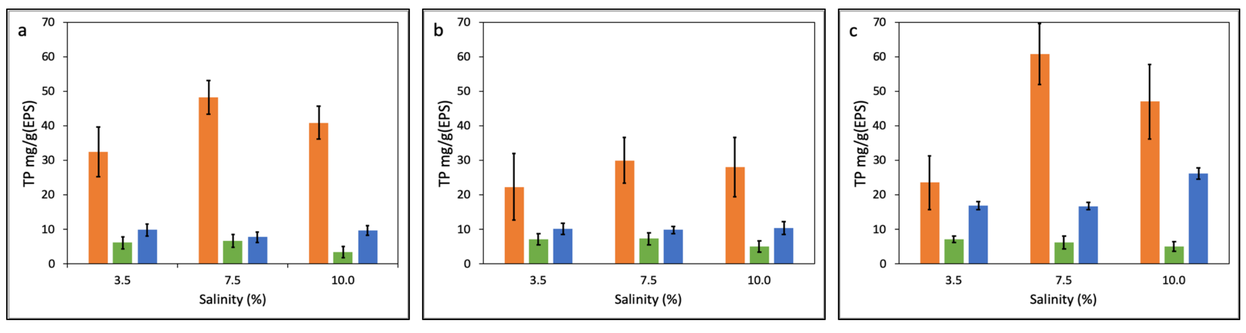


Figure 2: Total Protein of the EPS produced by mineral forming strains: a) DF112, b) DF2141 and non-mineral forming strain c) DF141 at different salinities and incubation temperatures of 20 ◦C, 30 ◦C and 40 ◦C.

Results

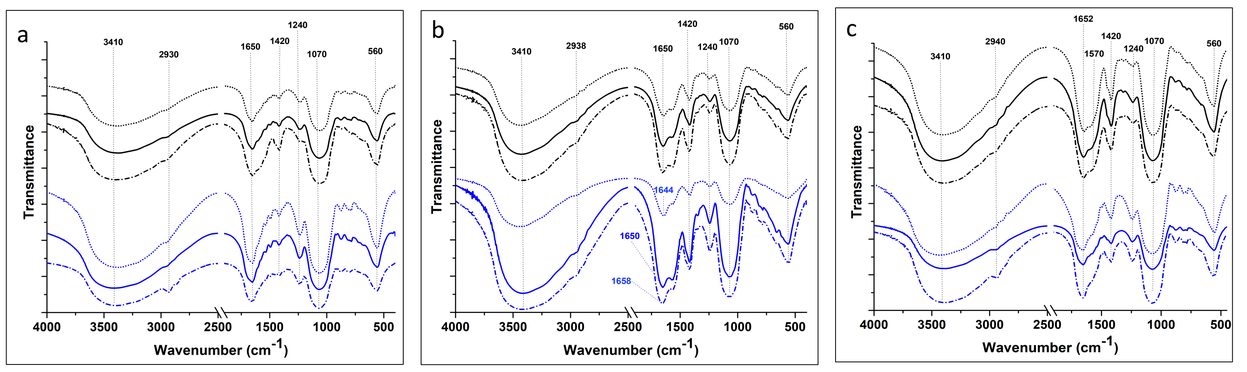


Figure 3: Representative FTIR spectra of the EPS Produced by mineral forming (blue lines) and non-mineral forming strains (black lines) at incubation temperature of a) 20 ◦C, b) 30 ◦C and c) 40 ◦C and salinity levels of 3.5%_ . . , 7.5%___ and 10%..... .

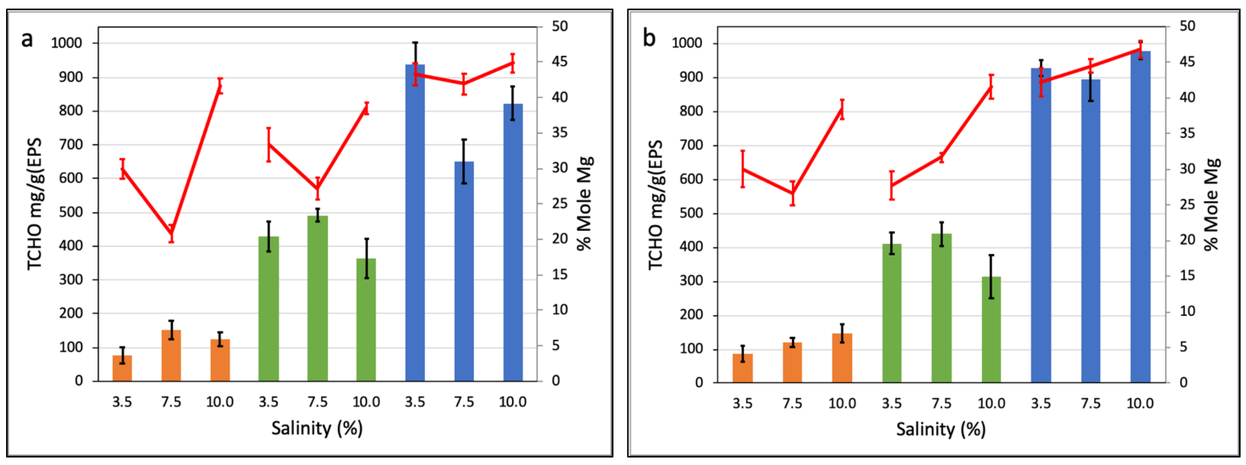


Figure 4 : Illustration of Total Carbohydrate content in the EPS produced by mineral forming strains a) DF112, b) DF2141 at different salinities (3.5%, 7.5% and 10%) and incubation temperatures of 20 ◦C, 30 ◦C and 40 ◦C (bars). The red lines represent the % mole Mg (± standard deviation) of the minerals formed at similar conditions.

Conclusion

- **The EPS associated with protodolomite are characterized by a high proportion of total carbohydrate (more than 75% of the total mass).**
- **FTIR of EPS produced by mineral-forming strains were distinct from those of the non-mineral-forming strain in areas associated with protein structures and areas associated with phosphoryl functional groups.**
- **Negatively charged carboxyl and/or hydroxyl groups favor —likely by dehydration of Mg— the incorporation of Mg into carbonate minerals.**
- **These results suggest that some organic molecules with specific functional groups may be of key importance for overcoming the kinetic barriers that else prevent the incorporation of Mg into carbonate minerals, a crucial step for the formation of dolomite in natural environment.**

References

Al Disi, Z. A. et al., 2019. Characterization of the Extracellular Polymeric Substances (EPS) of *Virgibacillus* strains capable of mediating the formation of high Mg-calcite and protodolomite. Marine Chemistry.

Al Disi, Z. A. et al., 2017. Evidence of a Role for Aerobic Bacteria in High Magnesium Carbonate Formation in the Evaporitic Environment of Dohat Faishakh Sabkha in Qatar. Frontiers in Environmental Science, Volume 5.

Arvidson, R. S. & Mackenzie, F. T., 1999. The dolomite problem; control of precipitation kinetics by temperature and saturation state. Am. J. Sci , Volume 1, pp. 257-288.

Bontognali et al., 2014. Microbially influenced formation of Mg-calcite and Ca-dolomite in the presence of Exopolymeric substances produced by sulphate-reducing bacteria. Terra Nova, Volume 26, pp. 72-77.

Braissant O. et al., 2003. Bacterially Induced Mineralization Of Calcium Carbonate In Terrestrial Environments: The Role Of Exopolysaccharides And Amino Acids. J. Sediment. Res., 73(3), p. 485–490.

Decho, A. W. & Gutierrez, T., 2017. Microbial Extracellular Polymeric Substances (EPSs) in Ocean Systems. Front Microbiol. 2017, 8(922), p. doi: [10.3389/fmicb.2017.00922].

Flemming et al., 2007. The EPS Matrix: The “House of Biofilm Cells”. Journal of Bacteriology, p. 7945–7947.

Roberts, J. A. et al., 2013. Surface chemistry allows for abiotic precipitation of dolomite at low temperature. PNAS, 110(36), p. 14540–14545

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