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Interactive comment on “Carbonate precipitation by the thermophilic archaeon *Archaeoglobus fulgidus*: a model of carbon flow for an ancient microorganism” by L. L. Robbins et al.

Anonymous Referee #2

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General comment

This paper deals with the precipitation of CaCO_3 in *Archaeoglobus fulgidus* cultures. The data presented here mainly rely on the use of isotopic markers (^{13}C and ^{14}C labeled compounds) to track the C through the biomineralization process.

Such data represent a important amount of work and are certainly of interest for many researcher working in the field of biogenic carbonates. However unclarities in the text and in the data presented make this article difficult to read. A good example of such unclarities can be found in figure 4. In this figure, supernatant and total DPM are not reported even if they are abundantly discussed in the text. Sample B in this figure is

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64 hours shorted than sample A (according to my understanding, sample have been treated exactly in the same way). In addition, it seems that not much effort were made to make this figure readable (see specific comments).

The references presented in this paper are pertinent with respect to the work done here. However most of the articles to which the authors refer are dating from the 90s. Many paper published within the last 10 years would also be pertinent in this paper. (see suggested references).

Finally, reading this paper I have been a bit frustrated. The data obtained using the ^{13}C isotopes are not much discussed. These data can probably bring more to the discussion. I am also surprised that dissolved organic carbon is not considered in this paper. However several studies have shown that *A. fulgidus* releases low molecular organic coumpounds and maybe EPS.

Specific comments

INTRODUCTION

The introduction section is well written and appealing. However as mentioned above, several references can be added.

P3410 L23-26: Microbial influences on carbonate precipitation include a variety of processes, such as the inhibition of extracellular carbonate nucleation via biologically produced molecules, the enhancement of carbonate precipitation due to cell surface charge distribution

There are plenty of references for this statement; for example:

Wright D. T., 1999, The role of sulfate-reducing bacteria and cyanobacteria in dolomite formation in distal ephemeral lakes of the Coorong region, South Australia.: *Sedimentary Geology*, v. 126, p. 147-157.

Wright D. T., and Oren A., 2005, Nonphotosynthetic Bacteria and the Formation of

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Carbonates and Evaporites Through Time: Geomicrobiology journal, v. 22, p. 27-53.

Verrecchia E. P., Freytet P., Verrecchia K. E., and Dumont J.-L., 1995, Spherulite in calcrete laminar crusts: biogenic CaCO₃ precipitation as major contributor to crust formation: J. Sedim. Research, v. A65, p. 690-700.

Bosak T., and Newman D. K., 2005, Microbial kinetic controls on calcite morphology in supersaturated solutions: J. Sediment. Res., v. 75, p. 190-199.

Braissant O., Cailleau G., Dupraz C., and Verrecchia E. P., 2003, Bacterially induced mineralization of calcium carbonate in terrestrial environments: the role of exopolysaccharides and amino acids: J. Sedim. Research, v. 73, p. 485-490.

Braissant O., Decho A. W., Dupraz C., Glunk C., Przekop K. M., and Visscher P. T., 2007, Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals: Geobiology, v. 5, p. 401-411.

Dupraz C., Visscher P. T., Baumgartner L. K., and Reid R. P., 2004, Microbe-mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas): Sedimentology, v. 51, p. 745-765.

Dupraz C., and Visscher P. T., 2005, Microbial lithification in marine stromatolites and hypersaline mats: TIM, v. 13, p. 429-438.

Ferris F. G., Phoenix V. R., Fujita Y., and Smith R. W., 2003, Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20°C in artificial groundwater: Geochimica Cosmochimica Acta, v. 67, p. 1701-1722.

Rivadeneira M. A., Parraga J., Delgado R., Ramos-Cormenzana A., and Delgado G., 2004, Biomineralization of carbonates by Halobacillus trueperi in solid and liquid media with different salinities: FEMS Microbial Ecology, p. 39-46.

Ercole C., Cacchio P., Botta A. L., Centi V., and Lepidi A., 2007, Bacterially Induced

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5, S1856–S1864, 2008

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Mineralization of Calcium Carbonate: The Role of Exopolysaccharides and Capsular Polysaccharides: Microscopy and Microanalysis, v. 13, p. 42-50.

Dupraz C., Visscher P. T., Baumgartner L. K., and Reid R. P., 2004, Microbe-mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas): Sedimentology, v. 51, p. 745-765.

Page 3411 L1: see comment above

Page 3411 L6: see comment above

Page 3411 L15-17: The authors emphasized sulfate reduction by *A. fulgidus*. They explain why sulfate reduction is particularly important with respect to the carbonate precipitation. Sulfate reduction is known to remove sulfate a kinetic inhibitor of precipitation (mainly dolomite formation). Sulfate reduction also increases alkalinity, thus favoring CaCO₃ precipitation. See:

Visscher P. T., and Stolz J. F., 2005, Microbial mats as bioreactors: populations, processes and products: Palaeo, v. 219, p. 87-100.

Wright D. T., and Wacey D., 2005, Precipitation of dolomite using sulphate-reducing bacteria from the Coorong Region, South Australia: significance and implications: Sedimentology, v. 52, p. 987-1008.

Bosak T., 2005, Laboratory models of microbial biosignatures in carbonate rocks [Ph. D. Thesis thesis]: Pasadena, CA, California Institute of Technology.

Bosak T., and Newman D. K., 2005, Microbial kinetic controls on calcite morphology in supersaturated solutions: J. Sediment. Res., v. 75, p. 190-199.

Page 3411 L19-20: *A. fulgidus* cells consist of an S-layer composed of glycoprotein subunits in hexagonal array (Stetter, 1988). Better say: cells surfaces consist.

MATERIAL METHODS

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5, S1856–S1864, 2008

Interactive
Comment

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Page 3412 L10-16: Since the publication added as a reference for the precipitation medium is a Ph.D. thesis and difficult to get, I would suggest to include the medium composition here.

Page 3412 L11: in 1-ml aliquot - remove

Page 3413 L18-28: OC is not introduced here (but much later in the results). It should be stated that OC stands for Organic pellet + Carbonates

Page 3414 L8-10: In order to determine whether CO₂ evolved from the oxidation of lactate by *A. fulgidus* was incorporated into precipitated carbonate minerals, experiments were conducted using [3-14 C] lactic acid as the primary source of carbon for the archaeon.

Does that mean that the medium contains other sources of carbon. If yes what is their amount / what ration do they represent. Obviously they can probably be neglected. However this must be stated.

Page 3415 L1-13: I guess a flow chart similar to the one made for figure 1 would be good to explain this experiment.

In addition it is not clearly stated how cells and carbonate are separated. I guess the method is similar to the one used for ¹³C.

RESULTS

Page 3416 L3-4: p-value and n should be indicated for the correlation.

Page 3416 L14-28: The description of the 3 different phases here is kind of confusing. It would really help to have them clearly indicated on figure 4, especially since the second addition of substrate re-induce the three phases (to my understanding). In addition the values for total and SN never appear, therefore it is difficult to agree or disagree with the authors. These data might be important with regards to the amount of DOC that is discarded with the supernatant when the samples are prepared (see

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general comment).

DISCUSSION

Page 3417 L18: incorporation of C in the form of CO₂. Better say: as CO₃²⁻ ions derived from the CO₂ produced by *A. fulgidus*. (or a similar sentence)

Page 3417 L20: same comment

Page 3417 L18- page 2418 L5: This part could probably be shortened. Although the metabolic information given in this section are important, it is not directly related to the data presented here. It just explains how the labeled compound are produced, not how they are incorporated or not in the carbonate minerals.

Page 3418 L24: Other works can be considered here. Several of this papers reports the precipitations on or associated with cells.

Bosak T. and Newman D. K. 2003. Microbial nucleation of calcium carbonate in the Precambrian. *Geology*, 31: 577-580.

Bosak T. and Newman D. K. 2005. Microbial kinetic controls on calcite morphology in supersaturated solutions. *J. Sediment. Res.*, 75: 190-199.

Van Lith Y., Warthmann R., Vasconcelos C. and McKenzie J. A. 2003. Sulfate-reducing bacteria induce low-temperature Ca-dolomite and high Mg-calcite formation. *Geobiology*, 1: 71-79.

Van Lith Y., Warthmann R., Vasconcelos C. and McKenzie J. A. 2003. Microbial fossilization in carbonate sediments: a results of the bacterial surface involvement in dolomite precipitation. *Sedimentology*, 50: 237-245.

Rivadeneira M. A., Delgado G., Ramos-Cormenzana A. and Delgado R. 1998. Biomineralization of carbonates by *Halomonas eurihalina* in solid and liquid media with different salinities: crystal formation sequence. *Res. Microbiol*, 149: 277-287.

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5, S1856–S1864, 2008

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Rivadeneira M. A., Delgado R., del Moral A., Ferrer M. R. and Ramos-Cormenzana A. 1994. Precipitation of calcium carbonate by *Vibrio* spp. from an inland saltern. *FEMS Microbial Ecology*, 13: 1997-204.

Rivadeneira M. A., Parraga J., Delgado R., Ramos-Cormenzana A. and Delgado G. 2004. Biomineralization of carbonates by *Halobacillus trueperi* in solid and liquid media with different salinities. *FEMS Microbial Ecology* 39:46.

Rivadeneira M. A., Ramos-Cormenzana A., Delgado G. and Delgado R. 1996. Process of Carbonate Precipitation by *Deleya halophila*. *Current Microbiology*, 32: 308-313.

Krumbein W. E. 1979. Calcification by bacteria and algae. In: Biogeochemical cycling of mineral-forming elements (Eds Trudinger P. A. and Swaine D. J.), pp. 47-68. Elsevier, Amsterdam.

Gautret P., Camoin G., Golubic S. and Sprachta S. 2004. Biochemical control of calcium carbonate precipitation in modern lagoonal microbialites, Tikehau atoll, French Polynesia. *J. Sediment. Res.*, 74: 462-464.

Gautret P. and Trichet J. 2005. Automicrites in modern cyanobacterial stromatolitic deposits of Rangiroa, Tuamotu Archipelago, French Polynesia: Biochemical parameters underlying their formation. *Sedimentary Geology*, 178: 55-73.

Ferris F. G., Shotyk W. and Fyfe W. S. 1989. Mineral formation and decomposition by microorganisms. In: Metal ions and bacteria (Eds Beveridge T. J. and Doyle R. J.), pp. 413-441. Wiley, New York.

Page 3419 L2 : Other references considering metal binding (especially calcium) may be considered here.

Perry T. D. IV., Klepac-Ceraj V., Zhang X. V., McNamara C. J., Polz M. F., Martin S. T., Berke N. and Mitchell R. 2005. Binding of harvested bacterial exopolymers to the surface of calcite. *Environ. Sci. Technol.*, 39: 8770-8775.

Ortega-Morales B. O., Santiago-Garcia J. L., Chan-Bacab M. J., Moppert X., Miranda-Tello E., Fardeau M. L., Carrero J. C., Bartolo-Perez B., Valad Gonzalez A. and Guezennec J. 2007. Characterization of extracellular polymers synthesized by tropical intertidal biofilm bacteria. *Journal of applied Microbiology*, 102: 254-264.

Braissant O., Decho A. W., Dupraz C., Glunk C., Przekop K. M. and Visscher P. T. 2007. Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals. *Geobiology*, 5: 401-411.

Beech I. B. and Cheung W. S. 1995. Interactions of exopolymers produced by sulphate-reducing bacteria with metal ions. *International Biodeterioration Biodegradation*, 35: 59-72.

Page 3419 L5-8: More effort can be made here to better describe the pictures from figure 2. A more extensive description should be included in fig. 2 caption or alternatively in the text. Arrows can be used to point cell remnant and CaCO_3 patches.

Page 3420 L11-13: major increase in metabolic activity. According to my experience, cell counting very often leads to strong variations (without standard deviation it is difficult to evaluate if such increase is really significant). In addition, in this case the activity is not monitored, only the cell count is. However since sulfate reduction tends to increase pH and alkalinity, the authors could link the pH values to the cell count to make their point stronger.

Page 3420 L26-28: p-value and n should be indicated

Page 3421-3422: section 4.2: To my knowledge *A. fulgidus* requires H_2 for the assimilation of CO_2 . As no H_2 was added to the culture in this experiment is this process really significant here ?

Page 3423 L14: 8218;evolved = lost or degassed ?

Page 3424 L8: better say: 8218;Áúnot sufficient to allow carbonate mineral stability

Page 3424 L16: start again. upon the second lactate amendment ? (in addition lactic acid may also lead to carbonate dissolution by chelating Ca ions).

Page 3426 L10: According to the acknowledgment, XRD analysis were performed. Is there any significant addition that can be made on the nature of the carbonate produced using these data ?... calcite, dolomite, vaterite,.

Fig. 1: OK

Fig. 2: OK, but requires a more detailed caption. This caption should include a description of the important stages. Since pictures are pretty small arrows could be added (cf comments)

Fig. 3: Using different scales for cell count/pH/biomass would make the graph more readable.

Fig. 4: This figures requires major editing (to make it pretty and more readable). In addition the figure should stick to the text, many elements described in the text do not appear in the figure

1) The four graphs should have the same size 2) Title should be removed and graph should be identified by letters (A,B,C,D) 3) Y axis should be the same for A,B and C,D (to allow comparison) 4) X axis should be the same for A,B and C,D (to allow comparison) 5) Phase I, II, III, should appear on the graph (grey areas for example) 6) SN and Total should appear 7) Remove grid 8) Include a caption which gives sufficient informations.

Interactive comment on Biogeosciences Discuss., 5, 3409, 2008.

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