

## GSA Data Repository item 2008159

## SUPPLEMENTARY METHODS

## Culture Experiments

Pure strains of *D. brasiliensis* were grown in 100 ml bottles with anoxic liquid medium maintained at 30°C in a thermostat-controlled incubator. The medium contained: 718 mM Cl<sup>-</sup>, 909 mM Na<sup>+</sup>, 9 mM Mg<sup>2+</sup>, 6.8 K<sup>+</sup>, 10 mM SO<sub>4</sub><sup>2-</sup>, 0.1 mM PO<sub>4</sub><sup>3-</sup>, 4.7 mM NH<sub>4</sub><sup>+</sup>, 0.007 mM Fe<sup>2+</sup>, 18.9 CO<sub>3</sub><sup>2-</sup>, 88.7 HCO<sub>3</sub><sup>-</sup>, 1 mM HS<sup>-</sup>, 7.3 mM CH<sub>3</sub>COO<sup>-</sup>, and 51.5 HCOO<sup>-</sup>. The pH was adjusted to 7.9 using NaOH. After 7 - 10 days of incubation, the Mg<sup>2+</sup> and Ca<sup>2+</sup> concentrations were increased through injection of a sterile anoxic solution. The final concentration of 80 mM Mg<sup>2+</sup> and 13 mM Ca<sup>2+</sup> correspond to that of Lagoa Vermelha (Warthmann et al., 2000), the natural environment from which *D. brasiliensis* was isolated (Warthmann et al., 2005). The progressive increase of Ca<sup>2+</sup> and Mg<sup>2+</sup> induces carbonate nucleation only when abundant EPS is present in the microbial culture. This allows for a better imaging of the early stage of mineral nucleation.

## Confocal Laser Scanning Microscopy (CLSM)

Samples, consisting of living hydrated flocks produced in *D. brasiliensis*, were investigated with CLSM the same day the anoxic culture experiments were stopped. Staining procedures were carried out considering methods and results described in previous studies (Decho and Kawaguchi, 1999; McSwain et al., 2005; Neu et al., 2001). To image EPS, the samples were stained with concanavalin-A labeled with tetramethylrhodamine (ConA-TMR). This lectin binds to  $\alpha$ -mannopyranosyl and  $\alpha$ -glucopyranosyl molecules and represents a versatile probe for detecting glyco-conjugates, which are major components of the EPS. To image bacterial cells, the samples were stained with fluorescein-5-isothiocyanate (5-FITC). This probe is not devised specifically for *Desulfovibrio* species, so possible contaminant organisms would also be detected. Carbonate minerals were visualized by reflected laser light. Parallel controls consisting of unstained samples, as well as carbonate globules bleached with a chlorine solution, allowed us to exclude important autofluorescence and non-specific stain. 5-FITC and ConA-TMR were purchased from Invitrogen. Staining solutions were prepared according to manufacturer instructions. 75  $\mu$ l of 5-FITC staining solution with a concentration of 10mg/ml was added to about 10 mg of hydrated sample immersed in 1 ml of sodium bicarbonate buffer. The working concentration of ConA-TMR was 100mg/ml with about 10 mg of hydrated samples immersed in 1 ml of staining solution. Incubation time was 30 minutes for 5-FITC and 15 minutes for ConA-TMR. Investigation of stained samples and control materials was carried out using a Leica TCS SP5. Observations were effectuated using a water immersion Leica HCX PL APO CS 63x lens (NA 1.2) with a resolution power of 162.7 nm in XY and 290.3 in Z (at 488nm). 5-FITC was excited by the 488 nm laser line and the fluorescence was detected between 530 - 555 nm. ConA-TMR was excited by the 496 nm laser line and emission was detected between 588 - 626 nm. Both fluorescence signals were recorded using a sequential mode. The 488 nm laser line was used as well for recording the reflected light channel (469 - 498 nm).

Image analyses were performed using Imaris software version 5.6. Figures 1A, B, and C represent maximum projections of stacks of two-dimensional sections. Z dimensions of the stacks are: 20  $\mu\text{m}$  (Fig. 1A), 18  $\mu\text{m}$  (Fig. 1B), and 26  $\mu\text{m}$  (Fig. 1C). Figure 1D represents a three-dimensional reconstruction from a cropped stack relating to Figure 1C.

### **Scanning Electron Microscopy With Cryogenic Preparation System (Cryo-SEM)**

Cryo-SEM observations and analyses of hydrated flocks formed in *D. brasiliensis* cultures were performed with a Philips XL-30 FEG scanning electron microscope equipped with an EDAX energy dispersive X-ray spectrometer (EDX). Cryofixation of the samples was carried out by immersion in a slush of liquid nitrogen at  $-210^{\circ}\text{C}$  followed by sublimation under vacuum within the SEM preparation chamber using a Gatan Alto 2500 cryotransfer system. During this process, water is transformed into ice with a crystalline domain size below 10 nm, which preserves the three-dimensional arrangement of even highly hydrated samples. Fresh surfaces were obtained by breaking the samples (cold fracture) with a blade installed within the preparation chamber, and subsequently platinum coated (7 nm). Images and EDX analysis presented in Figure 2 were obtained with a backscatter detector, an accelerating voltage of 15 kV, and a working distance of 10 mm.

### **Scanning Electron Microscopy (SEM)**

SEM observations and analyses of flocks formed in *D. brasiliensis* cultures were performed with a Zeiss supra 50 VP equipped with a EDAX energy dispersive X-ray spectrometer. Samples were washed in distilled water before being dried at room temperature. The washing step is crucial to avoid artifact morphologies, which may result with salt precipitation from the hypersaline medium used for the culture experiments. A 6 nm platinum coating was applied to the samples. The image and EDX analysis presented in Figure 3 were obtained with a backscatter detector, an accelerating voltage of 12 kV, and a working distance of 10 mm.

### **Supplementary References**

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