AGU PUBLICATIONS

Geochemistry, Geophysics, Geosystems

Supporting Information for

Prokaryote Communities at Active Chimney and In-Situ Colonization devices Modified after Magmatic Degassing Event (37°N MAR, EMSO-Azores Deep Sea Observatory

Céline Rommevaux[1,2], Pauline Henri[2], Jefferson Degboe[3], Valérie Chavagnac[3], Françoise Lesongeur[4], Anne Godfroy[4], Cédric Boulart[3,5], Christine Destrigneville[3], Alain Castillo[3]

[1]Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO UM 110, 13288, Marseille, France [2]Institut de Physique du Globe de Paris - Sorbonne Paris Cité, Université Paris Diderot, CNRS UMR 7154, Paris, France.

[3]GET UMR 5563 (CNRS/UPS/IRD/CNES), Université de Toulouse, Observatoire Midi-Pyrénées, Géosciences Environnement Toulouse, Toulouse, France.

[4]Univ Brest, CNRS, Ifremer, UMR 6197, Laboratoire de Microbiologie des Environnements Extrêmes, Plouzané, France.

[5]Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 7144, Adaptation et Diversité en Milieu Marin, Équipe Chimie Marine, Station Biologique de Roscoff, 29680 Roscoff, France

Contents of this file

Text S1
Figure S1 to S4
Tables S1 to S6

Additional Supporting Information (Files uploaded separately)

Introduction

Archaea and Bacteria 16S rRNA genes sequencing were detailed here.

Taxonomic affiliations were made using the SILVA database (v123) [*Quast et al.*, 2013]. The sequences with an affiliation bootstrap at the class level below 95% identity were considered as non-affiliated sequences. Sequence alignment, generation of the distance matrix from the aligned sequences and calculation of the rarefaction curves and richness indicators were performed with MOTHUR v.1.22.2 [*Schloss et al.*, 2009]. We defined Operational taxonomic units (OTUs) by a 0.03 distance level (*i.e.* sequences with ≥97% similarity are designated to a single OTU).

Tree topology and branch lengths were inferred using both the Neighbor-Joining method with the Maximum Composite Likelihood model [*Saitou and Nei*, 1987; *Tamura et al.*, 2004] and the Maximum Likelihood method based on the General Time Reversible model [*Nei and Kumar*, 2000] with MEGA software version 7 [*Kumar et al.*, 2016]. Maximum likelihood bootstrapping was carried out with 1,000 replicates.

Brut composition of gases and fluids were analyzed as described in the main paper.

Text S1.

Archaea and Bacteria PCR reactions and conditions for Sanger sequencing:

For Chim2008 and Chim2009 archaeal and Chim2008 bacterial 16S rRNA gene amplification, the PCR reaction mixture (50 µL) contained 10 µL 5x Go *Taq*® DNA polymerase buffer (Promega), 5µl $MgCl_2$ (25mM), 2 µL of 20mM GeneAmp® dNTPs (Invitrogen™), 0.2 µL of each primer at 100 pmol and 0.24 µL of 5 $U\mu L^{-1}$ GO *Taq*® DNA polymerase (Promega).

For LSTE1 and LSTE2 samples archaeal 16S rRNA genes amplification, the PCR reaction mixture (50 µL) contained 10 µL 5x Go *Taq*® DNA polymerase buffer (Promega), 1 µL of 10mM GeneAmp® dNTPs (Invitrogen™), 0.2 µL of each primer at 100 µM and 0.24 µL of 5 $U\mu L^{-1}$ GO *Taq*® DNA polymerase (Promega).

The protocol for Archaea DNA amplification was: (1) initial denaturation for 3 min at 94°C, (2) 30 cycles of 94°C (1 min), Tm (1 min 30 s) and 72°C (2 min), and (3) a final extension at 72°C for 6 min.

Because of the difficulty of extracting and amplifying DNA from chimney samples, the PCR product were purified using the Qiaquick® Spin Gel Extraction (Quiagen) following the manufacturer protocol, before cloning.

Bacteria PCR reactions and conditions for 454-pyrosequencing:

For LSTE1, LSTE2 and Chim2009 samples, 16S rRNA gene amplification, the PCR reaction mixture (25 µL) contained 2.5 µL 10x Platinum® *Taq* DNA polymerase Buffer (Invitrogen™), 0.75 µL of 50 mM $MgCl_2$, 0.5 µL of 10 mM GeneAmp® dNTPs (Invitrogen™), 0.75 µL of each primer at 100 µM and 0.15 µL of 1 $U\mu L^{-1}$ Platinum® *Taq* DNA Polymerase (Invitrogen™). All PCR reactions were carried out using an Applied Biosystems® 2720 or a Veriti® thermal cycler (Applied Biosystems®) with the following cycles: (1) initial denaturation for 3 min at 94°C, (2) 30 cycles of 94°C (30 s), 50°C (45 s) and 72°C (1 min 30 s), and (3) a final extension at 72°C for 10 min. The PCR products were visualized using gel electrophoresis.

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