

## Aerobic and anaerobic methane oxidation in terrestrial mud volcanoes in the Northern Apennines

C. Wrede<sup>a,c</sup>, S. Brady<sup>a</sup>, S. Rockstroh<sup>a</sup>, A. Dreier<sup>a,c</sup>, S. Kokoschka<sup>a</sup>, S.M. Heinzemann<sup>a</sup>, C. Heller<sup>b,c</sup>, J. Reitner<sup>b,c</sup>, M. Taviani<sup>d</sup>, R. Daniel<sup>a</sup>, M. Hoppert<sup>a,c,\*</sup>

<sup>a</sup> Institute of Microbiology and Genetics, Georg-August-University, Grisebachstr. 8, 37077 Goettingen, Germany

<sup>b</sup> Geoscience Centre Goettingen, Georg-August-University, Goldschmidtstr. 3, 37077 Goettingen, Germany

<sup>c</sup> Courant Centre Geobiology, Georg-August-University, Goldschmidtstr. 3, 37077 Goettingen, Germany

<sup>d</sup> ISMAR-CNR Bologna, Via Gobetti 101, 40100 Bologna, Italy

### ARTICLE INFO

#### Article history:

Received 8 December 2010

Received in revised form 10 June 2011

Accepted 12 June 2011

Available online 17 June 2011

#### Keywords:

Anaerobic oxidation of methane

ANME archaea

Mud volcano

Northern Apennines

### ABSTRACT

Methane oxidizing prokaryotes are ubiquitous in oxic and anoxic habitats wherever C<sub>1</sub>-compounds are present. Thus, methane saturated mud volcano fluids should be a preferred habitat of methane consuming prokaryotes, using the readily available electron donors. In order to understand the relevance of methane as a carbon and energy source in mud volcano communities, we investigate the diversity of prokaryotic organisms involved in oxidation of methane in fluid samples from the Salse di Nirano mud volcano field situated in the Northern Apennines. Cell counts were at approximately  $0.7 \times 10^6$  microbial cells/ml. A fraction of the microbial biomass was identified as ANME (anaerobic methanotroph) archaea by fluorescence *in situ* hybridization (FISH) analysis. They are associated in densely colonized flakes, of some tens of  $\mu\text{m}$  in diameter, embedded in a hyaline matrix. Diversity analysis based on the 16S rDNA genes, retrieved from amplified and cloned environmental DNA, revealed a high proportion of archaea, involved in anaerobic oxidation of methane (AOM). Aerobic methane-oxidizing proteobacteria could be highly enriched from mud volcano fluids, indicating the presence of aerobic methanotrophic bacteria, which may contribute to methane oxidation, whenever oxygen is readily available. The results imply that biofilms, dominated by ANME archaea, colonize parts of the mud volcano venting system.

© 2011 Elsevier B.V. All rights reserved.

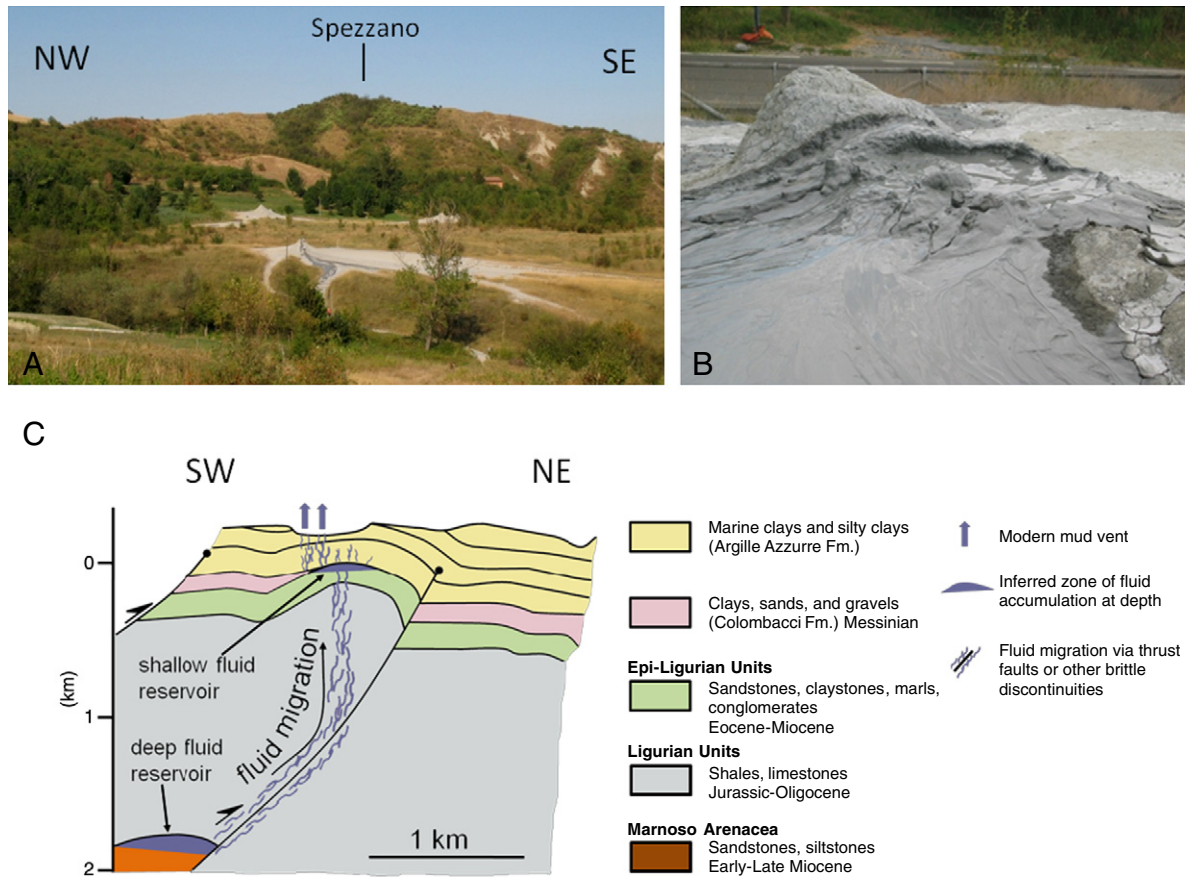
### 1. Introduction

Terrestrial mud volcanoes are peculiar geological structures, mostly associated with thrust belts, e.g. in the forelands of Alpine-Himalayan orogenic belt, namely in the Apennines, the Carpathians or the Caucasus (Dimitrov, 2002). Along the Apennines, these fluid venting structures could be traced back to the Oligocene (e.g. Taviani, 2001). Most of the recent mud volcanoes at the Emilia Apennines, but also elsewhere in Italy, are cold springs, expelling hydrocarbon-enriched, saline waters (Martinelli and Judd, 2004). The transported sedimentary material mostly leads to the formation of mud cones. At the Nirano mud volcano site (Fig. 1), fluid enriched mainly with clay fractions are transported to the surface, which leads to mud cones of several meters in height (Bonini, 2008). Analysis of the clasts and microfossils revealed that the fluids pass through the Pliocene-Pleistocene Argille Azzurre formation (marine silty clays) and the differently permeable Epi-Ligurian (Eocene-Miocene) and Ligurian (Cretaceous) units (Bonini, 2007 and references therein). Along

lithological boundaries of different permeability, overpressure or expansion will lead to the formation of fluid reservoirs, namely, along the Upper Marnoso Arenacas unit boundary (below the rather impermeable Ligurian unit) and along the Epi-Ligurian unit (below the impermeable fine grained sediments of the Argille Azzurre formation). Along the 2000 m-path between the deep fluid reservoir and the surface, a temperature difference of 20 °C per km has to be expected (della Vedova et al., 2001). Moreover, the upwelling pressurized fluid will be enriched with minerals from different geological units. It is likely that the fluids get also into contact with late Messinian Lago-Mare deposits, but possibly not with early Messinian evaporites (gypsum-rich). However, it may be expected that due to a hiatus readily at the Nirano mud volcano site, some of the fluids also pass directly the Epi-Ligurian/Argille-Azzurre boundary (Fig. 1 C). This feature may also be reflected by geochemical data, collected from different mud cones over time, showing a certain variability of the mineral concentrations in the fluid, especially sulfate (Martinelli and Judd, 2004; Duchi et al., 2005; this study). Since mud volcanoes are in contact to deep fluid reservoirs (s. above), they represent a window into the deep biosphere (in our case the deep terrestrial biosphere) with microbial communities that are apparently just distantly related to adjacent (e.g. soil) habitats (cf. Alain et al.,

\* Corresponding author at: Institute of Microbiology and Genetics, Georg-August-University, Grisebachstr. 8, 37077 Goettingen, Germany. Tel.: +49 551 393832.

E-mail address: [mhopper@gwdg.de](mailto:mhopper@gwdg.de) (M. Hoppert).



**Fig. 1.** Geological setting of the Nirano mud volcano field. A. Panoramic view of the Nirano mud volcano field towards NE. B. Active mud cone analyzed in this study. C. Geological cross section in the direction of the view axis of (A) (adapted from Bonini, 2008).

2006; Nacke et al., 2011). Besides carbon dioxide and perhaps reduced sulfur compounds or transition metals, it is obvious that methane must be an important source for carbon as well as electrons in microbial processes of mud volcanoes. Hence, the prevalence of methane-oxidizing organisms should be expected. Under anaerobic conditions, in the presence of electron acceptors (in particular sulfate), anaerobic oxidation of methane is likely.

Different groups catalyzing AOM have been identified yet. Most studies are based on 16S rRNA and *mcrA* genes, supported by lipid biomarker fingerprints with stable isotope analysis (Knittel and Boetius, 2009). Hitherto, three ANME clusters were identified: ANME-1, ANME-2 with the subclasses -2a, -2b, and -2c, and ANME-3. All clusters belong to the Euryarchaeota, but they are not monophyletic. While ANME-1 phylotypes are distantly related to Methanomicrobiales (Hinrichs et al., 1999), ANME-2 and ANME-3 are related to Methanosarcinales (Orphan et al., 2001; Niemann et al., 2006). A further group has been described as ANME-2 d or GoM Arc I. This group is not monophyletic with the other ANME-2 subgroups (Mills et al., 2005; Lloyd et al., 2006; Martinez et al., 2006). ANME-1 and ANME-2 are the most diverse groups detected in a multitude of habitats. ANME-3 and GoM Arc I have been, so far described for a few habitats.

As metabolic pathway for AOM reversed methanogenesis has been proposed as most likely. Several lines of evidence support this hypothesis. In particular, the depletion of  $^{13}\text{C}$  in the archaeal lipids, the demonstrated uptake of methane by ANME-2 and the presence of nearly all genes relevant for the methanogenic pathway in ANME-1 (Hinrichs et al., 1999; Orphan et al., 2001; Hallam et al., 2004; Meyerdierks et al., 2010). Though sulfate has been recognized as the electron acceptor for methane oxidation, sulfate reduction is not

carried out within this pathway, since enzymes of sulfate reduction are missing. An unknown reduced intermediate or an electron transport mechanism must be involved for transfer of reducing equivalents to syntrophic partners. The diversity of ANME subgroups and the different structure of the archaeal/eubacterial consortia implies also diverse ways how reducing equivalents may be transferred between the symbionts. ANME-1 archaea were found in microbial mats in loose association with SRB of *Desulfococcus/Desulfosarcina* (DSS) group (Reitner et al., 2005a, 2005b). ANME-2 archaea are also associated with members of the DSS group (Boetius et al., 2000), but  $\alpha$ - or  $\beta$ -proteobacterial partners and *Desulfobulbus*-related genera were described as well (Perntaler et al., 2008). Remarkably, ANME-2 without contact to syntrophic partners could also be observed (Orphan et al., 2002; Knittel and Boetius, 2009).

Recently, AOM with iron and manganese as electron acceptors in enrichment cultures was described. These electron acceptors would provide an energy yield by a factor of 10 higher than in sulfate dependent AOM. Though it has been suggested that ANME-1 and ANME-3-related archaea are involved, it remains unknown if the electron acceptors have a direct or indirect influence on the process (Beal et al., 2009).

In contrast, the pathway of aerobic oxidation, conducted by several eubacterial groups, is well understood. Type I methanotrophs belong to the subdivision of  $\gamma$ -proteobacteria and are divided in 10 genera. Among them, *Methylobacter*, *Methylomicrobium* and *Methylomonas* are most well studied. The hitherto type I genera *Methylococcus* and *Methylocaldum* are also referred to as type X methanotrophs, which are distinguished by certain physiological, biochemical and phylogenetic characteristics (Bowman, 2006; McDonald et al., 2008). The group of type I methanotrophs use the ribulose monophosphate (RuMP)

Download English Version:

<https://daneshyari.com/en/article/4689789>

Download Persian Version:

<https://daneshyari.com/article/4689789>

[Daneshyari.com](https://daneshyari.com)