

MICROBIAL COMMUNITY STRUCTURE OF DEEP SEA CARBONATE CRUSTS: NOVEL ARCHAEA INVOLVED IN ANAEROBIC METHANE OXIDATION ?

Sander K. Heijs^{1*}, Giovanni Aloisi², Ioanna Bouloubassi³, Larry J. Forney⁴, Richard D. Pancost⁵, Catherine Pierre², Jaap S. Sinninghe Damsté⁶, Jan C. Gottschal¹ and the MEDINAUT shipboard scientific party[#]

¹ University of Groningen, Department of Microbiology, Center of Ecological and Evolutionary Studies, AA Haren, The Netherlands
s.k.heijs@biol.rug.nl

² Laboratoire d'Océanographie Dynamique et de Climatologie, Université Pierre et Marie Curie, Paris, France.

³ Laboratoire de Physique et Chimie Marines, Université Pierre et Marie Curie, Paris, France

⁴ Department of Biological Sciences, University of Idaho, USA

⁵ Organic Geochemistry Unit, School of Geochemistry, University of Bristol,

⁶ Netherlands Institute for Sea Research, Department of Marine Biogeochemistry and Toxicology, AB Den Burg (Texel), The Netherlands

Keywords: Eastern Mediterranean, carbonate crust, bacteria, deep sea ecology

Three carbonate crust samples collected from distant mud volcanoes in the eastern Mediterranean were used in a multidisciplinary study to gain insight in the processes that lead to carbonate crust formation and the role of anaerobic methane oxidation in these processes. The combination of lipid biomarker analysis and microbial community structure studies based on analyses of 16S rRNA gene sequences identified a highly diverse methane-consuming archaeal community composed mostly of novel species. The results obtained, suggest that the ability to oxidise methane under anoxic conditions by archaea may be phylogenetically more widespread within the Archaea than previously suggested.

Methane is an important greenhouse gas and recent geologic and glacial studies have shown its potential impact on climate change (1). As a consequence, controls on methane production and consumption are important considerations in the discussion on climate change. Authigenic carbonate crusts are sinks for methane carbon originating from depth or shallow gas hydrates (2,3). Understanding their formation by anaerobic methane oxidation is important in assessing the contribution of ocean-atmosphere carbon exchanges to global warming.

In the eastern Mediterranean, seven mud volcanoes (1600 and 2000 m depth) were explored with the *Nautilus* submersible during the Medinaut cruise of the R/V *Nadir* in 1998. Their central parts actively seep methane to the bottom waters and are covered by thick carbonate crust pavements (4). In a multidisciplinary study, three carbonate crust samples from distant mud volcanoes (MN16BT2, MN14BT3 and MN12BT4, respectively) were used to study the processes that lead to carbonate crust formation and to detect microbes involved in anaerobic methane oxidation. Special emphasis was placed on the possible role of anaerobic microorganisms in this process. To this end the microbial community structure was analysed (16S rRNA studies) and results were combined with lipid biomarker analysis and compound-specific carbon isotope measurements.

Analyses of cellular lipid biomarkers showed the presence of diverse and novel isoprenoidal alkyl diethers, tetraethers and irregular isoprenoids that are diagnostic of archaea (5,6) in crusts MN16BT2 and MN13BT4, whereas MN14BT3 showed little abundance of such markers. These compounds were strongly depleted in ¹³C (-101‰ < δ¹³C < -69‰) indicating assimilation, rather than production, of ¹³C-depleted methane by Archaea (7). In addition, lipid biomarkers indicative for sulphate reducing bacteria (SRB) were detected in all three crusts. These SRB biomarkers were also depleted in ¹³C. These low δ¹³C-values of cellular lipid biomarkers and the co-occurrence of SRB with Archaea are consistent with the hypothesis that anaerobic methane oxidation is carried out by consortia of these prokaryotes (8,9).

DNA appeared to be present in large amounts in the crusts. Analyses of the microbial community structure based on 16S rDNA sequences showed the presence of bacterial and archaeal communities in crusts MN16BT2 and MN13BT4. In crust MN14BT3 16S rDNA of members of an archaeal community were not detected. All three crusts showed little similarity between the bacterial communities. These bacterial communities consisted of phylogenetically diverse organisms, most of which appear to be novel species. Based on 16S rDNA analysis, only crust MN16BT2 showed the presence of organisms related to sulphate reducing bacteria (β-proteobacteria).

Analysis of the archaeal community of crust MN16BT2 revealed the presence of three archaeal clusters, two of which appear to be related to Archaea previously identified in cold seep sediments (10). One

of these two clusters has been suggested to be involved in anaerobic methane oxidation (10). The third cluster comprised novel, previously undetected archaeal sequences. In crust MN13BT4, two clades, consisting of ten new archaeal species comprising several lineages were identified. All these clusters in crust MN13BT4 are distinct from those of crust MN16BT2 and have not been previously reported for any setting.

The combination of lipid biomarker analysis and microbial community structure studies indicated the presence of a highly diverse methane-consuming archaeal community composed of mostly novel species. The occurrence of anaerobic methane oxidation by archaeal communities both in the absence and presence of SRB may suggest that the ability to anaerobically oxidise methane may be phylogenetically more widespread within the Archaea than previously suggested.

References

- 1- Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.-M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V.M., Legrand, M., Lipenkov, V.Y., Lorius, C., Pépin, L., Ritz, C., Saltzman, E., and M. Stievenard, 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature*, 399, 429-436.
- 2- Ritger, S., Carson, B. and E. Suess, 1987. Methane-derived authigenic carbonates formed by subduction-induced pore-water expulsion along the Oregon/Washington margin. *Geol. Soc. Am. Bull.*, 98, 147-156.
- 3- Bohrmann, G., Greinert, J., Suess, E. and M. Torres, 1998. Authigenic carbonates from the Cascadia subduction zone and their relation to gas hydrate stability. *Geology*, 26, 647-650.
- 4- MEDINAUT/MEDINETH Shipboard Scientific Parties, 2000. Linking Mediterranean brine pools and mud volcanism. *EOS Transactions*, American Geophysical Union, 81, 625-632.
- 5- Kates, M., 1993. pp.261-295. In: Kates, M., Kushner, D.J. and Matheson, A.T. eds., *The biochemistry of Archaea (Archaeobacteria)*. Elsevier Science, New York.
- 6- Hopmans, E.C., Schouten, S., Pancost, R.D., van der Meer, M.T.J. and J. Sinninghe Damsté, 2000. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, 14, 1-5.
- 7- Pancost, R.D., Sinninghe Damsté, J.S., de Lint, S., van der Maarel, M.J.E.C., Gottschal, J.C. and MEDINAUT Shipboard Scientific Party, 2000. Biomarker evidence for widespread anaerobic methane oxidation in Mediterranean sediments by a consortium of methanogenic archaea and bacteria. *Appl. Environ. Microbiol.*, 66, 1126-1132.
- 8- Hoehler, T.M., Alperin, M.J., Albert, D.B., and C.S. Martens, 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: Evidence for a methanogen-sulfate reducer consortium. *Glob. Biogeochem. Cycle*, 8, 451-463.
- 9- Boetius, A., Ravensschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B.B., Witte, U., and O. Pfannkuche, 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, 407, 623-626.
- 10- Hinrichs, K.-U., Hayes, J.M., Sylva, S.P., Brewer, P.G., and E.F. DeLong, 1999. Methane-consuming archaeobacteria in marine sediments. *Nature*, 398, 802-805.

J. L. Charlou, J. P. Donval, A. Fiala-Medioni, J.-P. Foucher, R. Haese, P. Henry, G. de Lange, J. Mascle, G. Nobe, H. Pelle, M. Sibuet, M. J. E. C. van der Maarel, J. M. Woodside