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ScienceDirect
Physics and Chemistry of the Earth 32 (2007) 232–240
PHYSICS and CHEMISTRY of the EARTH
www.elsevier.com/locate/pce

Detection and cultivation of indigenous microorganisms in Mesozoic claystone core samples from the Opalinus Clay Formation (Mont Terri Rock Laboratory)

L. Mauclaire [a,*], J.A. McKenzie [a], B. Schwyn [b], P. Bossart [c]

[a] *Laboratory of Geomicrobiology, Geological Institute, ETH-Zentrum, 8092 Zurich, Switzerland*
[b] *NAGRA, Hardstrasse 73, 5036 Wettingen, Switzerland*
[c] *Geotechnical Institute, Fabrique de Chaux, 2882 Sainte Ursanne, Switzerland*

Received 1 July 2005; received in revised form 23 November 2005; accepted 30 December 2005
Available online 12 October 2006

Abstract

Although microorganisms have been isolated from various deep-subsurface environments, the persistence of microbial activity in claystones buried to great depths and on geological time scales has been poorly studied. The presence of in-situ microbial life in the Opalinus Clay Formation (Mesozoic claystone, 170 million years old) at the Mont Terri Rock Laboratory, Canton Jura, Switzerland was investigated. Opalinus Clay is a host rock candidate for a radioactive waste repository. Particle tracer tests demonstrated the uncontaminated nature of the cored samples, showing their suitability for microbiological investigations. To determine whether microorganisms are a consistent and characteristic component of the Opalinus Clay Formation, two approaches were used: (i) the cultivation of indigenous micoorganisms focusing mainly on the cultivation of sulfate-reducing bacteria, and (ii) the direct detection of molecular biomarkers of bacteria. The goal of the first set of experiments was to assess the presence of cultivable microorganisms within the Opalinus Clay Formation. After few months of incubation, the number of cell ranged from 0.1 to 2×10^3 cells ml^{-1} media. The microorganisms were actively growing as confirmed by the observation of dividing cells, and detection of traces of sulfide. To avoid cultivation bias, quantification of molecular biomarkers (phospholipid fatty acids) was used to assess the presence of autochthonous microorganisms. These molecules are good indicators of the presence of living cells. The Opalinus Clay contained on average 64 ng of PLFA g^{-1} dry claystone. The detected microbial community comprises mainly Gram-negative anaerobic bacteria as indicated by the ratio of iso/anteiso phospholipids (about 2) and the detection of large amount of β-hydroxy substituted fatty acids. The PLFA composition reveals the presence of specific functional groups of microorganisms in particular sulfate-reducing bacteria (*Desulfovibrio*, *Desulfobulbus*, and *Desulfobacter*). This study demonstrates that microorganisms are a characteristic component of the unperturbed Opalinus Clay Formation.

Keywords: Biomarker; Fatty acid; Extremophile; Subsurface; Radioactive waste; Sulfate-reducing bacteria

1. Introduction

The discovery of extensive active microbial populations beneath the deep ocean floor (Mauclaire et al., 2004) and continental subterranean environment (Fredrickson et al., 1997; Pedersen, 1996) has far reaching implications for our understanding of the influence of microbial metabolism on life, Earth's evolution and subsurface environmental problems. In these extreme environments, previously thought to be uninhabited, reside highly adapted bacteria that play a very important role in geochemical cycles (Fredrickson and Onstott, 1996; Haveman and Pedersen, 2002; Parkes et al., 2000; Pedersen, 1996). The study of the deep biosphere demands attention from the scientific community because of specific environmental problems associated with our modern society.

Sustainable management of radioactive waste is one of these problem, which all countries using nuclear power as

* Corresponding author.
E-mail address: laurie.mauclaire@erdw.ethz.ch (L. Mauclaire).

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doi:10.1016/j.pce.2005.12.010

energy source will need to resolve. Disposal in the deep subsurface has been suggested as an ultimate solution for low- to high-level radioactive waste and argillaceous formations are widely considered to be suitable as a geological barrier for radioactive waste disposal. Several countries are currently investigating the suitability of various clay formations for this purpose. In Switzerland, the Mont Terri research program is dedicated to investigate the storage ability of the lower Dogger Opalinus Clay Formation, which is considered as a potential repository host rock for radioactive waste repository.

Microbial activity, which might have both positive and negative impacts on repository conditions, needs to be closely investigated to assess the safety of subsurface radioactive disposal. Recent experiments under conditions similar to those in a high-level radioactive water repository demonstrated that a fraction of the microbial community can survive for several months within the compacted clay used as repository buffer (Pedersen et al., 2000; Stroes-Gascoyne et al., 1997, 2002), and that bacteria can persist over 35 million years within the natural clay barrier (Boivin-Jahns et al., 1996).

The goal of this work was to assess the presence of autochthonous microorganisms entombed in the 170 million years old Opalinus Clay Formation of the Mont Terri Rock Laboratory. Geochemical approach based on the detection of phospholipid fatty acids (PLFA) was used to assess the presence of living cells (White et al., 1979). Furthermore, cultivation experiments were conducted in order to investigate the potential revival of autochthonous bacteria.

2. Material and methods

2.1. The Opalinus Clay Formation and sample collection

The Opalinus Clay Formation (Dogger, Aalenian, 170 million years old) was formed as a marine sediment consisting of fine mud particles (Table 1). Porewater squeezed from the Opalinus Clay Formation is highly mineralized, and consists of sodium-chloride water with a total dissolved solid up to 20 g/l (Table 2). This water contains significant component of seawater that is million years old (Pearson et al., 2003).

Table 1
Characteristics of the Opalinus Clay (shaly facies)

	Value	Reference
Mineralogy	Silicates (illite, illite-smectite mixed layers, chlorites, kaolinites, albites, K-feldspar), carbonate (calcite, dolomite, ankerite, siderite) and quartz	Mazurek (1999)
Water content	4–12%	Thury and Bossart (1999)
Hydraulic conductivity	$<1 \times 10^{12}$ m/s	Bossart et al. (2002)
Porosity	12–18%	Thury and Bossart (1999)
Pore size	0.01–0.02 μm	Mais (1998)

Table 2
Composition of the Mont Terri interstitial water (Pearson et al., 2003) compared with seawater (Turkian, 1969) and the microbial media used for the culture experiment

	Formation water	Seawater	Microbial media	
			SRB95	SRB87
pH	7.96	8.1	7.2	7.2
Sodium	245	470	378	416
Potassium	1.1	10.1	7.3	1.4
Calcium	15.2	10.3	0.9	1.3
Magnesium	17.1	53.8	29.3	4.2
Ammonium	0.57		5.6	5.6
Chloride	287	546	390	359
Sulfate	13.7	28.2	23.3	21.1
Phosphorus			7.3	1.2
HCO_3^-	0.8	2.25	22.2	59.5
TOC	1.1	0.04	14	31
DL-Lactic acid				39
Na_2-DL-Malate			20	
Yeast extract (g)			0.5	

Concentrations in mmol per l (except yeast extract in g).

The presence of autochthonous microorganisms in the Opalinus Clay Formation was assessed within two locations distant about 80 m from the undeformed shaly facies. The first core (Core A, 12 m long, 84 mm inner diameter) was air-drilled in the HE-D niche on December 2003. The second core (Core B, 15 m long) was aseptically drilled in the PP niche on February 2004 (Daumas, 2004). 9 m were drilled with compressed air, and the last 6 m were drilled with compressed nitrogen. From 9 to 12 m, the triple core technique provided samples with a diameter of 76 mm in a thin walled steel tube. From 12 to 15 m, as for the air-drilled 9 m, the usual double core technique was applied and provided samples with a core diameter of 84 mm in a plastic envelope.

After the core recovery, samples were packed aseptically and anaerobically. Cores were transported under ambient temperature to the laboratory where the experiment started within the next 24 h. All the apparatus were carefully washed and sterilized by either autoclaving or flaming with ethanol.

2.2. Contamination test

To assess the microbial contamination risk, fluorescent microspheres (0.4 (+/−0.001) μm in diameter, Polysciences, Inc.), the same approximate size of microorganisms, were used as particulate tracers during the drilling of Core A (Colwell et al., 1992). The concentration of microsphere was calculated to simulate the equivalent of 10^4 times the potential contamination due to the presence of bacteria in the compressed air. Microspheres were deployed in latex bag containing 10 ml of suspension of