Integrated Ocean Drilling Program
Expedition 337

Deep Coalbed Biosphere off Shimokita: Microbial Processes and Hydrocarbon System Associated with Deeply Buried Coalbed in the Ocean

Keywords: deep biosphere, methane hydrate, coalbed, microbial processes, CO_2 sequestration, archaea, bacteria, prokaryotes

Fumio Inagaki
Kochi Institute for Core Sample Research
Japan Agency for Marine-Earth Science and Technology
Monobe B200, Nankoku
Kochi 783-8502
Japan

Kai-Uwe Hinrichs
MARUM-Center for Marine Environmental Sciences and Department of Geosciences
University of Bremen
Leobener Str.
D-28359 Bremen
Germany

Yu'suke Kubo
Expedition Project Manager
Center for Deep Earth Exploration
Japan Agency for Marine-Earth Science and Technology
3173-25 Showa-machi, Kanazawa-ku
Yokohama 236-0001
Japan

Abstract
Among the least characterized geobiological systems on Earth that can be accessed by scientific ocean drilling are deeply buried hydrocarbon reservoirs in sedimentary formations along continental margins. In particular, the microbiological and abiotic processes associated with deeply buried coalbed in the ocean remain poorly understood: e.g., what role does subsurface microbial life play for the formation of hydrocarbon reservoirs? Do deeply buried hydrocarbon reservoirs act as geobiological reactors that sustain microbial life by releasing nutrients and energy sources? Do the conversion and transport of hydrocarbons and other reduced compounds influence biomass, diversity, activity and functionality of deep subseafloor microbial populations? What are the fluxes of both thermogenically and biologically produced organic compounds and how important are these for the carbon budgets in the shallower subsurface and the ocean? To address these important scientific questions, IODP Expedition 337 will drill and study the deep coalbed biosphere off the Shimokita Peninsula in the northwestern Pacific using *Chikyu*. This expedition aims to extend the maximum penetration of scientific ocean drilling to over 2,200 mbsf and will explore the microbial ecosystem associated with a deeply buried coalbed, i.e., a habitat that had never been accessed by previous scientific ocean drilling. The riser drilling and operational strategy will involve spot-coring in representative intervals throughout the hole, continuous mud gas logging (gas composition and stable isotope), continuous geochemical and sedimentological analysis of cuttings retrieved during riser drilling, wireline logging from 647 to 2,200 mbsf including sampling of pristine formation fluids, and pressure core sampling in methane hydrate bearing strata in an adjacent hole under the *in-situ* pressure condition. The cored intervals in the deep hole will be subject to intense sampling for shipboard and shore-based investigations. In addition, we will address scientific problems related to CO_2 sequestration in offshore coalbed formations through *ex-situ* experiments using the recovered core materials. The latter experiments will investigate, among others, how supercritical CO_2 interacts with minerals, organic matter and life in the deep subsurface and examine how CO_2 storage may affect biogeochemical carbon cycling. Ultimately, IODP Expedition 337 will greatly advance our knowledge of the coalbed subseafloor hydrocarbon system and the deep biosphere, including its CO_2 sequestration potential.

Schedule for Expedition 337

Expedition 337 is based on Integrated Ocean Drilling Program drilling proposal number 745CPP-Full "Coal-Bed Hydrocarbon System and Deep-Biosphere: Geobiology of Deep Carbon Cycles and Implications for CO_2 Sequestration Potentials" (available soon at CDEX website www.jamstec.go.jp/chikyu/eng/). Following ranking by the IODP Scientific Advisory Structure, the expedition was scheduled for the *DV Chikyu*, operating under contract with the Center for Deep Earth Exploration (CDEX). The expedition 337 is currently scheduled to depart the port of Hachinohe, Japan on 15th March, 2011 and return to the same port on 21st May, 2011. A total of 68 days will be available for the drilling, coring, and downhole measurements described in this report (for the current detailed schedule, see www.iodp.org/expeditions. Further details on the facilities aboard *Chikyu* and CDEX can be found at www.jamstec.go.jp/chikyu/eng/. Supporting site survey data for Expedition 337 are archived in the IODP Site Survey Data Bank.

Expedition 337 is the first expedition originating from a Complimentary Project Proposal (CPP) in IODP history. Platform operational cost for this expedition, as well as the development of sampling and analytical facilities on *Chikyu* such as Hybrid-PCS, radioisotope and mud gas-monitoring container laboratories, is covered by the Strategic Fund for Strengthening Leading-edge Research and Development from the Japan Society for the Promotion of Science (JSPS), MEXT, Japan (FY2010-2011, PI: Fumio Inagaki, JAMSTEC).

Introduction

Marine subsurface hydrocarbon reservoirs and the associated microbial life in continental margin sediments are among the least characterized Earth systems that can be accessed by scientific ocean drilling. Our knowledge of the biological and abiotic processes associated with hydrocarbon production is limited, because of the highly limited opportunities to conduct scientific ocean drilling initiatives using deep-riser coring in natural gas and oil fields. A number of fundamental questions regarding deep subseafloor hydrocarbon systems remain unanswered. For example:

- What role does subsurface microbial life play for the formation of hydrocarbon reservoirs?
- Do the deeply buried hydrocarbon reservoirs such as methane hydrates and terrestrial coalbeds act as geobiological reactors that sustain life by releasing nutrients and carbon substrates?
- Do the conversion and transport of hydrocarbons and other reduced compounds influence biomass, diversity, activity and functionality of deep subseafloor microbial populations?
- What are the fluxes of both thermogenically and biologically produced organic compounds and how important are these for the carbon budgets in the shallower subsurface and the ocean?

To address these important scientific questions, IODP Expedition 337 will drill and study the hydrocarbon system associated with deeply buried coalbed off the Shimokita Peninsula in the NW Pacific by coring down to 2,200-2,500 mbsf using the riser-drilling system of *Chikyu* (Fig. 1). Based on the existing seismic profiles as well as previous natural gas-drilling surveys, it has been demonstrated that thick Eocene to Cretaceous lignite layers (~60% TOC, approximately 100 m in

thickness) are present under an Oligocene unconformity layer, hosting large quantities of coalbed methane (CBM) with the production rate of ~210 x 10^3 m^3/day (Osawa et al., 2002). During the *Chikyu* shakedown expedition CK06-06 in 2006, methane hydrates were observed in porous ash and sand layers of shallow subsurface sediments down to 350 mbsf. In the recovered sediments, counts of living microbes exceeded 10^7 cells/cm^3 and were thus highly abundant compared to other continental margin locations (Morono et al., 2009) (Fig. 2). This project aims to extend the drilling depth down to 2,200 mbsf and explore the microbial ecosystem associated with the deep coalbed in oceanic sediments, that is, a habitat that has not been accessed by previous scientific ocean drilling. Hence, the study area is an ideal natural subsurface laboratory to study the deep carbon cycle and the deep biosphere.

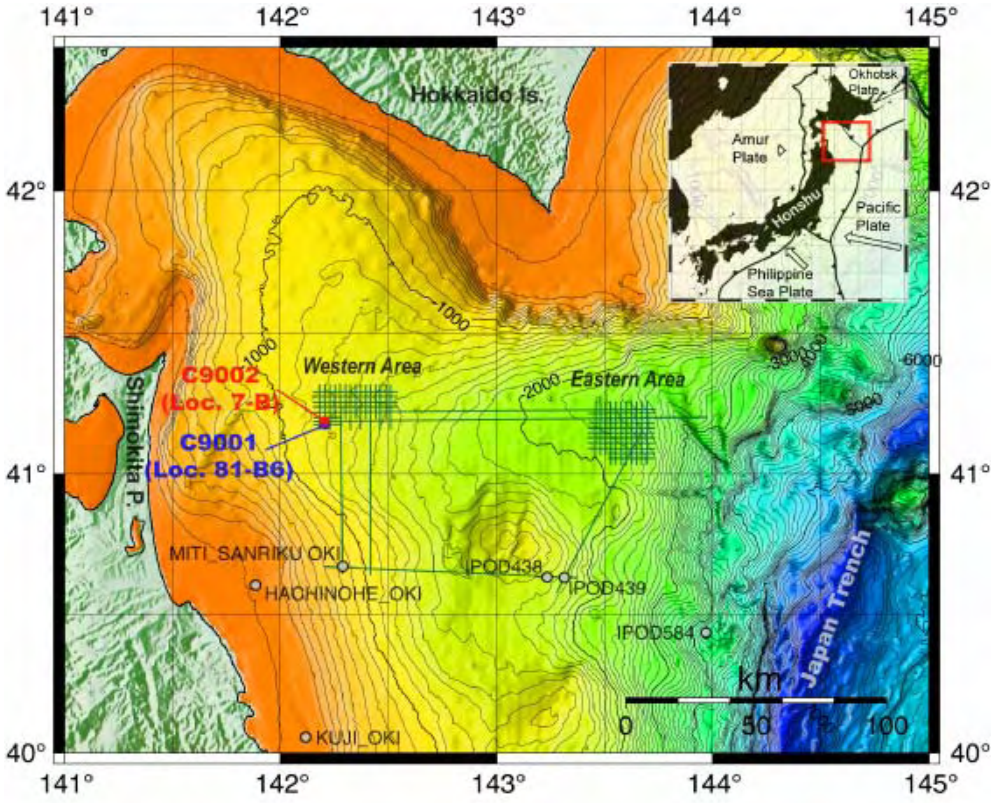

Fig. 1. Index map of off-Shimokita Drilling Test area with bathymetry, seismic survey track lines and locations of existing drill holes. HPCS coring locations, C9001 and C9002, drilled in late November 2005, are also indicated. Inset map exhibits a plate configuration around Japanese Islands and the location of the index map with red open square.

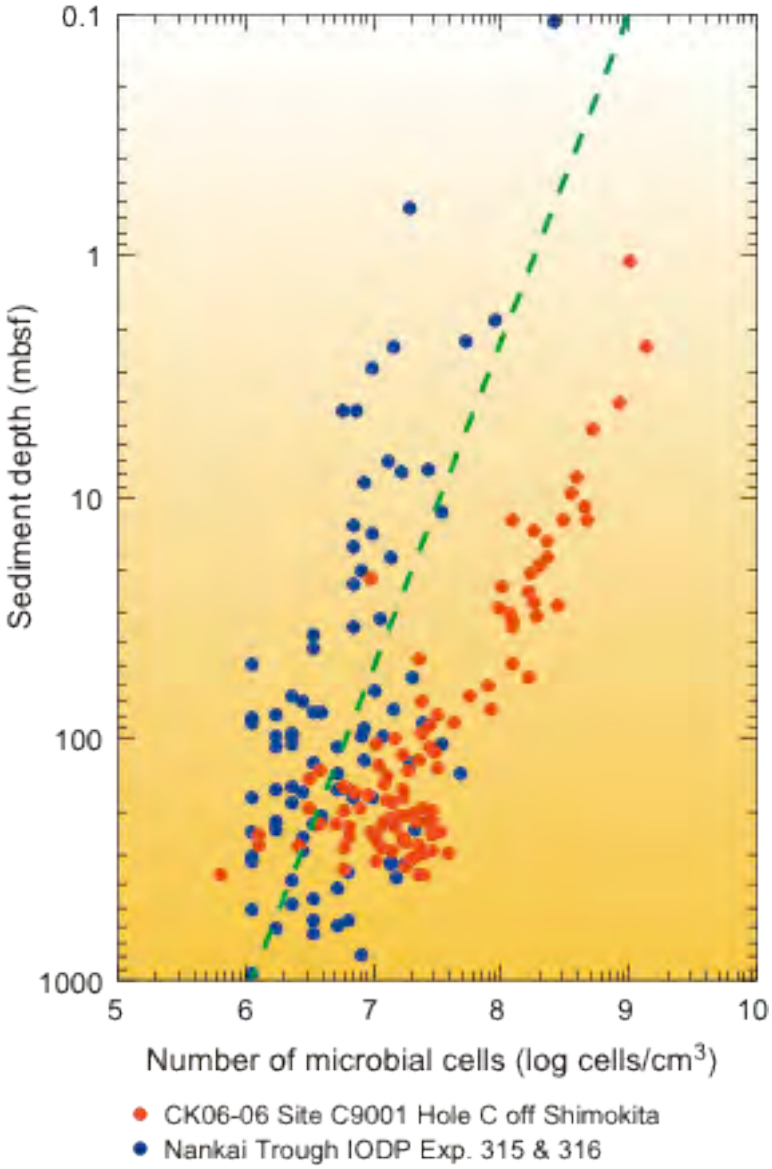

Fig. 2 Microbial cell abundance in marine subsurface sediments. Red and blue points indicate cell abundance in sediments cored from offshore the Shimokita Peninsula and the Nankai Trough seismogenic zone, respectively. The plot data were obtained by the image-based cell enumeration technique (Morono et al., 2009). Green line shows the global average of microbial abundance in continental margin sediments evaluated by acridine orange direct count (Parkes et al., 2000).

Deep subseafloor life in continental margin sediments

Subseafloor sediments harbor a remarkably sized microbial biosphere that constitutes approximately 10-30% of total living biomass on our planet (Parkes et al., 1994, 2000; Whitman et al., 1998; Lipp et al., 2008; D'Hondt et al., 2009). To date, microbial cells have been observed in sediments ranging in age to the Cretaceous and in subsurface depth to 1,626 mbsf (Newfoundland Margin, ODP Leg 210: Roussel et al., 2008). Diagenetic models of pore water chemical constituents as well as ^{14}C- and ^{35}S-radiotracer incubation experiments showed that metabolic activities of deep subseafloor microbes are extremely low due to the low supply of energy-rich substrates (D'Hondt et al., 2002, 2004) but are often stimulated at geochemical and/or lithological interfaces such as porous

ash layers and sulfate methane transition zones (Inagaki et al., 2003; Parkes et al., 2005; Biddle et al., 2006; Sørensen and Teske 2006). The metabolic activities of subseafloor microbial communities are controlled by the flux of bio-available electron donors and/or acceptors, some of which are derived either from the overlying seawater via photosynthetic primary productions (D'Hondt et al., 2004, 2009; Lipp et al., 2008) or from crustal fluids underlying sedimentary habitat (Cowen et al., 2003; Nakagawa et al., 2006; Engelen et al., 2008). Fluid flow regimes in the subseafloor environment control availability of energy to microbial life. Hence, the geologic and sedimentological characteristics represent crucial factors controlling habitability of the deep subsurface. Culture-independent molecular ecological surveys of 16S rRNA gene fragments reveal that the microbial communities in continental margin sediments are predominantly composed of species lacking cultivated relatives such as the bacterial members within the candidate division JS1, Chloroflexi and Planctomycetes as well as the archaeal members within the Deep-Sea Archaeal Group (DSAG), the Miscellaneous Crenarchaeotic Group (MCG) and the South African Gold Mine Euryarchaeotic Group (SAGMEG) (e.g., Inagaki et al., 2003, 2006a; Inagaki & Nakagawa, 2008; Inagaki, 2010). The carbon isotopic analysis of intact polar lipids (IPLs) and fluorescent *in-situ* hybridization (FISH)-stained cells suggest that sizeable populations of heterotrophic Archaea significantly contribute to microbial biomass in organic-rich sediments, even at the sulfate-methane transition zone where the occurrence of anaerobic oxidation of methane mediated by methanotrophic archaea and sulfate reducing bacteria takes place (Biddle et al., 2006). Quantitative analysis of the IPLs extracted from sediments (>1 mbsf) at a variety of oceanographic settings reveal that approximately 87% of IPLs are archaeal, suggesting that the previous DNA-based PCR experiments had significant biases affecting the extraction and quantification (Lipp et al., 2008; Teske and Sørensen 2008). Despite the significance of heterotrophic microbes in biogeochemical cycling within the continental margin sediments, the metabolic characteristics of organic matter degradation and fluxes of secondary metabolites remain largely unknown (e.g., Hinrichs et al., 2006).

Coal diagenesis: Microbiological significance for biogeochemical cycles

Within the generally energy-starved deep subseafloor biosphere, the lignite coal is a conceivable source of nutrients and energy for deep microbial communities. Microbiological and geochemical studies of terrestrial coal deposits and subsurface aquifers suggest that microorganisms play important ecological roles in coal diagenesis, resulting in substantial quantities of CBM as a terminal product (Brown et al., 1999; Detmers et al., 2001; Fry et al., 2009; Krüger et al., 2008; Shimizu et al., 2007; Strapoc et al., 2008) (Fig. 3). The microbial communities in terrestrial coaly habitats are phylogenetically highly diverse with relatively low cell density of less than 10^6 cells cm^{-3}. For example, methane-producing archaea (i.e., methanogens) such as the genera *Methanoculleus*, *Methanobacterium*, *Methanolobus*, and *Methanosarcina*, as well as some potential acetate-producing bacteria (i.e., acetogens) such as *Acetobacterium* were predominant in a deep borehole aquifer directly connected to the coal deposits in Hokkaido Island of Japan (Shimizu et al., 2007). Using incubation tracer experiments and FISH technique, it was found that active methanogenesis occurs even in a highly altered graphite deposit (Krüger et al., 2008). Very recently, Fry et al. (2009) reported sizable cultivable populations of potential sulfate reducing bacteria, methanogens, acetogens, and lignite-utilizing heterotrophs in the uplifted coaly sediments of northern New Zealand based on results from the most probable number (MPN) cultivation method. Metabolic activities were stimulated at the geologic interfaces between coal and sand/silt layers as reported from other terrestrial deep subsurface

black shales (e.g., Krumholz et al., 1997), and the concentrations of organic acids in the coal layers were higher than in normal deposits, consistent with the co-occurrence of coal diagenesis and microbial processes.

Despite the microbiological and (bio-)geochemical significance of coaly deposits for the global carbon cycle, there have been no studies of coal layers that are deeply buried in the subseafloor, mainly because of the safety regulations related to hydrocarbon gas-related hazards during non-riser drilling. In continental margin sediments, large quantities of gaseous hydrocarbons as well as H_2, CO, CO_2, organic acids, aromatic compounds, NH_4, N_2, sulfur compounds, etc., are potentially generated via thermogenic and/or biogenic degradation processes from the coaly deposit. All of these compounds are potential nutrient and energy sources that support redox reactions mediated by the deep subseafloor microbial communities. Hence, coalbeds and their active microbial life may influence the upward transport of dissolved gases and organic matter in geo-fluids as well as the accumulation of gas hydrates in overlying sediments. In this regard, the connectivity between deep subsurface microbial activity and the formation and deposition of gas hydrates is a frontier research theme in geobiology and geochemistry that can only be studied by a dedicated initiative as IODP Expedition 337.

Exploring the feasibility of CO_2 sequestration in deep offshore geological repositories

To date, the CO_2 capture and sequestration (CCS) into deep subsurface environments such as oil, gas and porous aquifers is considered as a solution for reducing the emission of substantial amounts of anthropogenic CO_2 and preventing dangerous consequences of the anticipated future climate change. CCS offshore deep subseafloor environments has a number of advantages, including a positive risk assessment compared to shallow water CCS (Schrag, 2009). It has been predicted that CCS can potentially reduce future world emissions from fuel energy by 20% (Dooley et al., 2006). However, the behavior and stability of CO_2 as well as its chemical reactions in deep subsurface repositories are still largely uncertain.

In this project, we will address the multiple scientific issues of the geological CO_2 sequestration through *ex-situ* experimentations using the cored materials, tackling the following fundamental questions:

How does the liquid or supercritical CO_2 spatially penetrate into the various lithostratigraphic settings? How does the CO_2 react with minerals, organic matter and with life in the deep subsurface? What are the impacts of long-term CO_2 storage on biogeochemical carbon cycling and the subsurface biosphere on different time scales? Conducting various multidisciplinary *ex-situ* experimental studies using the cored materials as well as *in-situ* logging characterizations of the deep riser-hole, Exploration 337 will significantly expand our knowledge of the coalbed subseafloor hydrocarbon system, including the physiochemical and biological factors that determine the potential for CO_2 sequestration.

Background

Drilling Site: Off Shimokita Peninsula

In 2002 and 2003, two-dimensional seismic surveys off Shimokita Peninsula were carried out in a 15 km (N–S) x 30 km (E–W) area by *R/V Polar Duke* and *Polar Princess*. During the NT04-01

cruise using *RV Natsushima* in 2003, the detailed bathymetry mapping was performed using SeaBat 8160 Multibeam Echosounder with a frequency of 50 kHz (Taira and Curewits, 2005) (Fig. 3). Site C9001 is located on the cross point of seismic lines ODSR03–BS and ODSRW03–H81. During the *Chikyu* shakedown cruise CK06-06 in 2006, 365 meters of sediment cores were recovered from the upper sedimentary section at Site C9001 (41°10.5983' N, 142°12.0328' E, 1180 m water depth), approximately 80 km off the coast of Shimokita Peninsula of Japan (Figs. 1 and 3)(Aoike, 2007). Riser drilling was also tested down to 647 mbsf without coring at Site C9001, 20" casings were installed down to 511 mbsf, and then the riser hole was suspended for the future riser drilling opportunity. Given those pilot surveys, the geologic resistance and potential safety hazards for the riser drilling operations at Site C9001 have already been evaluated as feasible.

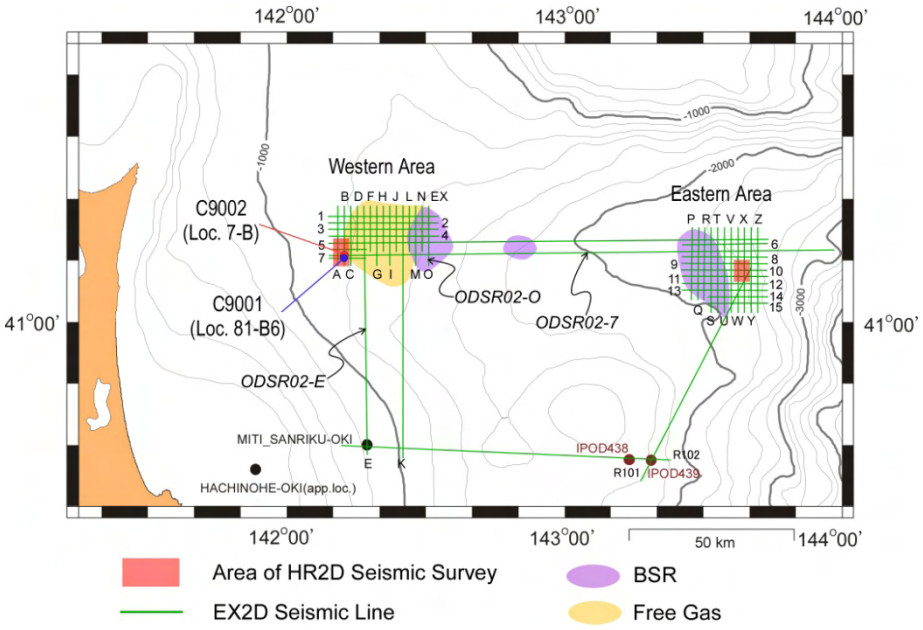

Fig. 3. Location map of seismic survey tracklines and existing drill holes. Red solid squares indicate areas for High Resolution 2D seismic survey. BSR is recognized at about 600 mbsf in the western area and 640 mbsf in the Eastern Area. In addition, existence of free gas is anticipated in the Western Area, adjacent to the BSR-developed zone.

Geological setting

The drilling site C9001 is located in a forearc basin formed by the subduction of the Pacific Plate (~8 cm/year, WNW plate motion vector: Seno et al., 1994) beneath northeastern Honshu, Japan (Fig. 1). The Hidaka Trough, a sedimentary basin formed by subsidence in the drilling area, originates just offshore southwest of Hokkaido and extends to the Japan Trench. Along the coastal area of the Shimokita Peninsula, both sedimentary and volcanic rocks younger than late Cretaceous lie scattered on Triassic to early Cretaceous sedimentary rocks or Cretaceous granites.

Several scientific drilling expeditions have been carried out off Shimokita Peninsula: IPOD Legs 56 and 57 in 1977, IPOD Leg 87 in 1982, and ODP Leg 186 in 1999. In addition, well data are available from hydrocarbon drilling explorations carried out between 1977 and 1999 (Japan Natural Gas Association and Japan Offshore Petroleum Development Association, 1992; Osawa et al., 2002). Seismic profiles around Site C9001 show pull-up blanking reflections below bottom-simulating reflectors (BSRs) at around 360 mbsf, suggesting the occurrence of methane hydrates and a strong upward flux of free hydrocarbon gases (Fig. 4). A thick and prominent Quaternary

sedimentary unit on-laps to a Pliocene unit and is thought to be composed mainly of alternating beds of mud and sand with intercalations of thin volcanic tephras and locally-developed gravel/sand layers. The Pliocene unit consists primarily of alternating beds of mudstone and sandstone. Below these relatively recent formations, there are sedimentary deposits ranging from Cretaceous to Miocene in age that are cut by many landward-dipping normal faults. The presence of thick coal layers was confirmed by the natural gas drilling exploration at Site MITI Sanriku-Oki, approximately 50 km southward from Site C9001 (Fig. 1; Osawa et al., 2002). Sonic logging data in the MITI Sanriku-Oki well showed that three major tuff layers involving coal layers with 30 m, 45 m, and 80 m thickness (40-60% TOC in lignite coal layer and 0.5-2% TOC in tuffs) are present in Eocene and Pliocene-upper Cretaceous horizons, in which vitrinite reflection values (Ro) were in the range between 0.5 and 0.7, indicating relatively immature coal (Osawa et al., 2002). The *in-situ* temperatures are well within the range of the habitable zone of microbes, based on the reported thermal gradient of 22.5°C/km (Osawa et al., 2002).

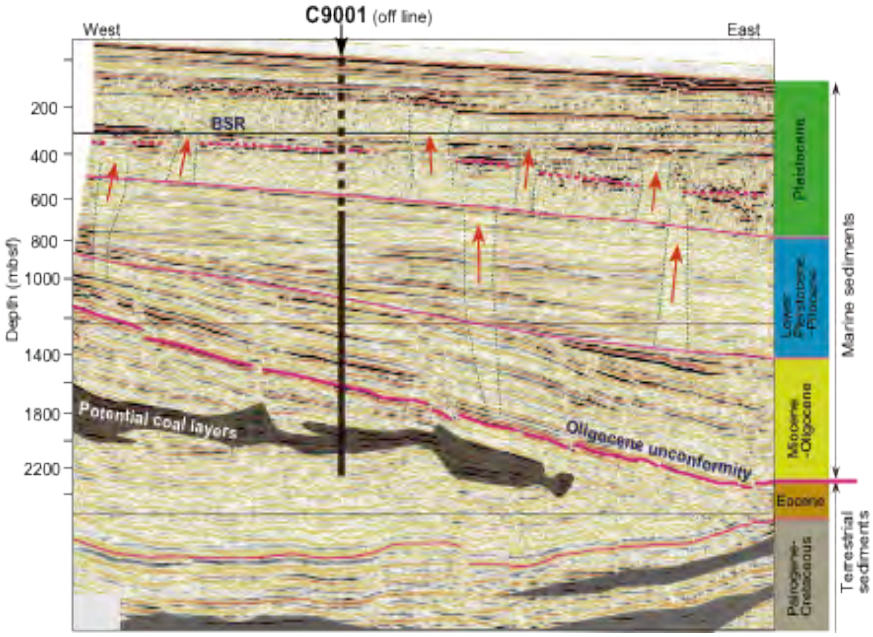

Fig. 4. Seismic profile in the vicinity of Site C9001 off Shimokita Peninsula. The potential coal-bearing layers and the fluid and gas plumes are indicated by the dark grey zones and the green dotted lines with red arrows, respectively. The picked lines represent the predicted age of stratigraphic boundaries including the Oligocene unconformity.

Preliminary scientific results from shallow sedimentary column at Site C9001

The cored sediments from Site C9001 during the *Chikyu* shakedown cruises were composed primarily of diatom-rich hemi-pelagic silty clay intercalated with volcanic tephras and sand layers (Fig. 5). Preliminary biostratigraphic age models indicate very high sedimentation rates, ranging from 54 to 95 cm kyr^{-1}, and an approximate core-bottom age of 640 ka (Aoike, 2007; Aoike et al., 2010; Domitsu et al., 2010). During the CK06-06 *Chikyu* shakedown cruise, core temperature anomalies were monitored immediately by Thermo-View IR camera after recovery in order to identify and locate methane hydrates. We observed methane hydrate formations (Fig. 6) as well as microbial aggregates (Fig. 7) in porous ash and sandy layers. Geochemical analyses of interstitial waters consistently showed that the chloride concentrations (and other sea salts) were notably

depleted within the porous layers as a result of hydrate dissociation (Tomaru et al., 2009). Iodine concentrations and radio-isotopic compositions ($^{129}I/I$) of the deep pore waters suggest that the iodine and oldest hydrocarbon sources could be as old as 40 Ma. Acetate concentrations in pore waters were over 100 μmol L^{-1}throughout the sediment column (maximum 313 μmol L^{-1}), which is presumably related to coal diagenesis in deeper zone (Yoshioka et al., unpublished data).

A newly developed cell counting technique using a computer image showed that the cored sediments contained abundant microbial cells with counts over 10^7 cm^{-3} down to 365 mbsf (Morono et al., 2009); these counts were approximately two orders of magnitude higher than those in sediments from the Nankai Trough seismogenic zone (i.e., microbiological samples from IODP Expeditions 315 and 316: see Fig. 2). The abundance of Bacteria and Archaea was studied by quantitative real-time PCR and slot-blot hybridization techniques, suggesting a significant contribution of Archaea to the subseafloor microbial biomass (average 40% at DNA level) (Lipp et al., 2008).

The metabolic activity of organiclastic sulfate reduction, sulfate reduction coupled with anaerobic oxidation of methane (AOM), aceticlastic methanogenesis, and autotrophic (CO_2 reducing) methanogenesis rates were investigated using ^{35}S and ^{14}C radiotracers, showing high AOM activity below the sulfate-methane interface and relatively low methanogenic activity throughout the core column (Inagaki et al., unpublished data). Using a sediment sample from C9001, carbon and nitrogen incorporation rate of deep subseafloor microbes was studied at single-cell level using nano-scaled secondary ion mass spectrometry (NanoSIMS) (Morono et al., in prep.). A large fraction of subseafloor microbes was found to incorporate ^{13}C- and ^{15}N-labeled substrates into the biomass, and their metabolic rates provide *in-vitro* evidence for energy-starvation.

Cultivation of aerobic and anaerobic microorganisms has been conducted and a variety of microbes and their enzymatic activities were observed in the core sediments (Kobayashi et al., 2008). Using a continuous-down flow bioreactor system, the cored sediments were anaerobically incubated at 10 °C. Synthetic seawater containing glucose, yeast extract, acetate and propionate as energy sources was supplemented into the bioreactor. After 289 days operation, significant methane production was observed (Imachi et al., in prep.). The $\delta^{13}C_{CH4}$ value was approximately -80‰, strongly suggesting the occurrence of microbial methanogenesis.16S rRNA and its gene-based clone analyses of the bioreactor enrichment culture revealed that phylogenetically diverse microbes were cultivated in the bioreactor system and the dominating phylotypes were closely related to the typical environmental phylotypes that have been frequently observed in various subseafloor zones. Predominant archaeal members enriched in the reactor were affiliated with the methanogens such as the genera *Methanobacterium*, *Methanoccoides* and *Methanosarcina*, and the uncultured archaeal lineages (Fig. 8). Several attempts transferring into traditional batch-type cultivations successfully led to the isolation of several methanogens and anaerobic microbes. These provide direct evidence for the presence of metabolically active and cultivable microbial populations in subseafloor habitats off Shimokita Peninsula.

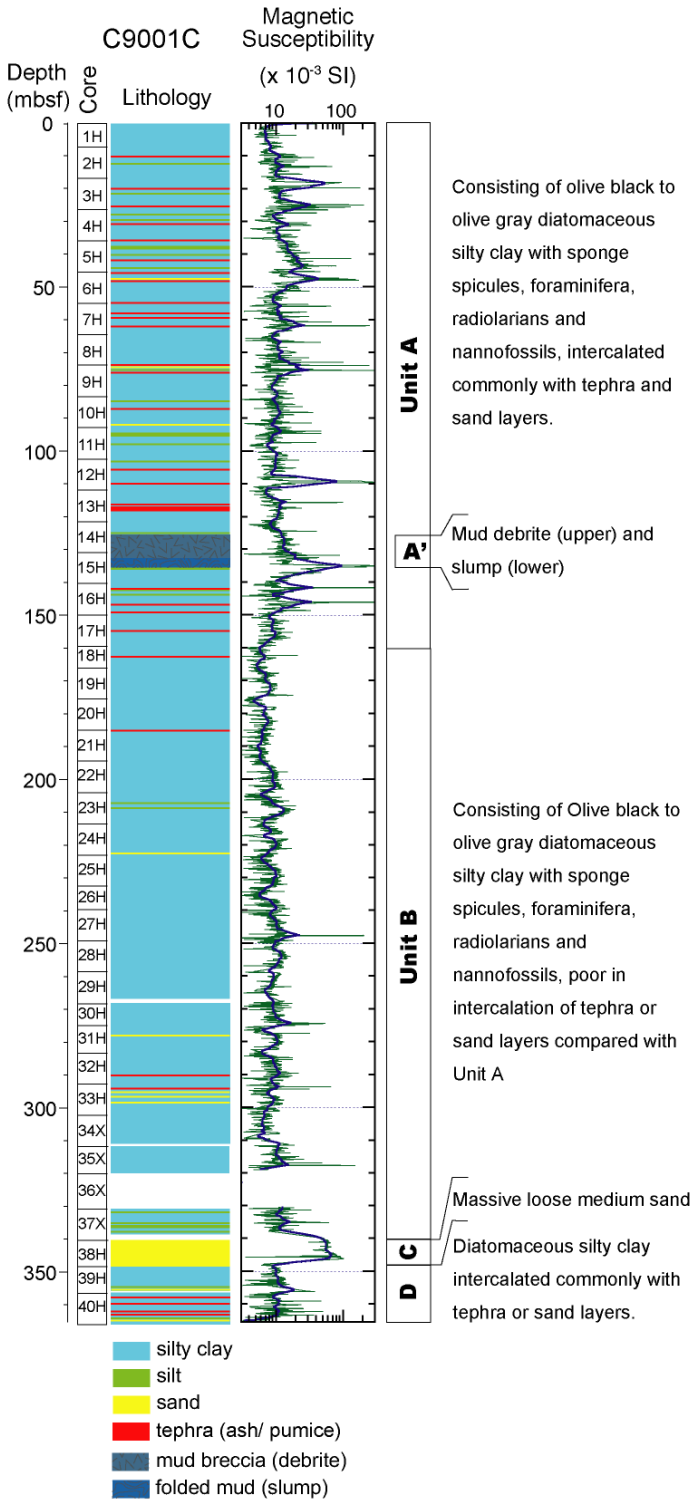

Fig. 5. Lithostratigraphy of 365-meter sediment core from Site C9001, Hole C, recovered during the *Chikyu* shakedown expedition in 2006.

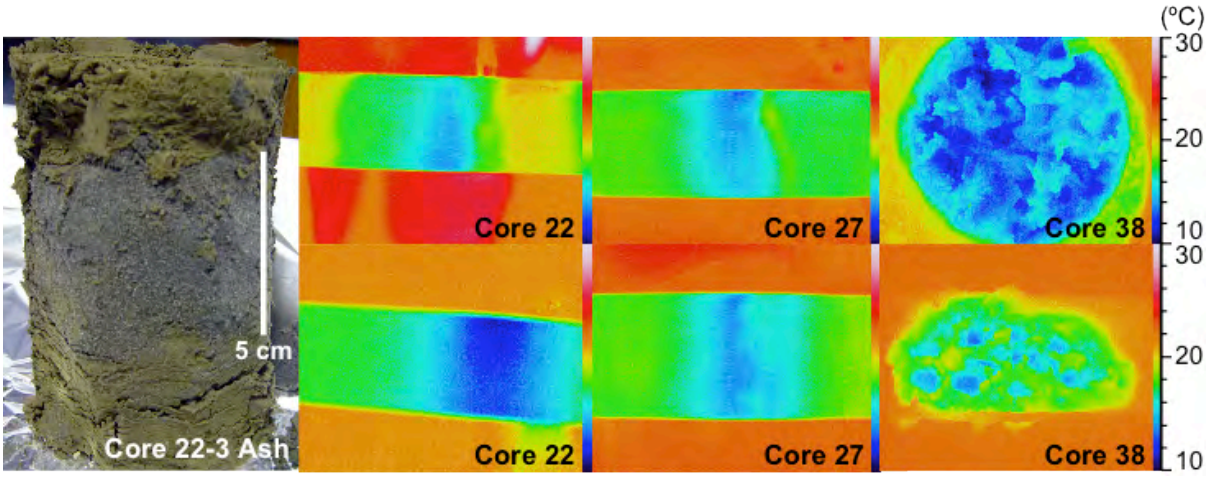

Fig. 6. Detection of methane hydrate at Site C9001 by monitoring negative temperature anomalies using Thermo-View camera.

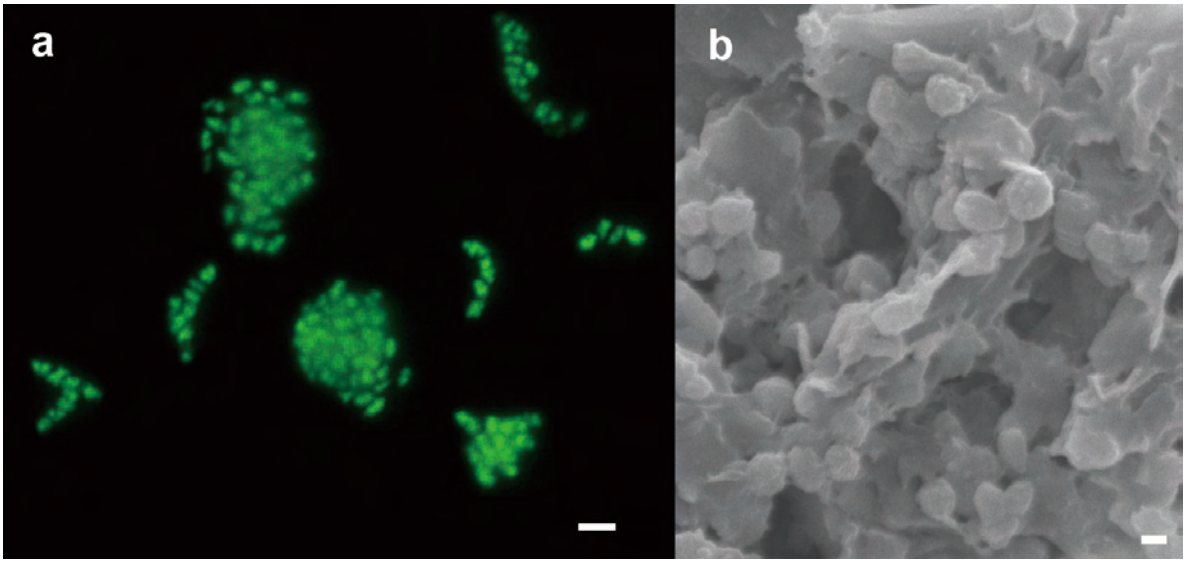

Fig. 7. Microbial cells detected from methane hydrate-bearing sediments at 346 mbsf, Site C9001. (a) Fluorescent microscopic image of SYBR Green I-stained cell aggregate. Bar: 2μm. (b) Scanning electron micrograph of cell aggregates. Bar: 200 nm.

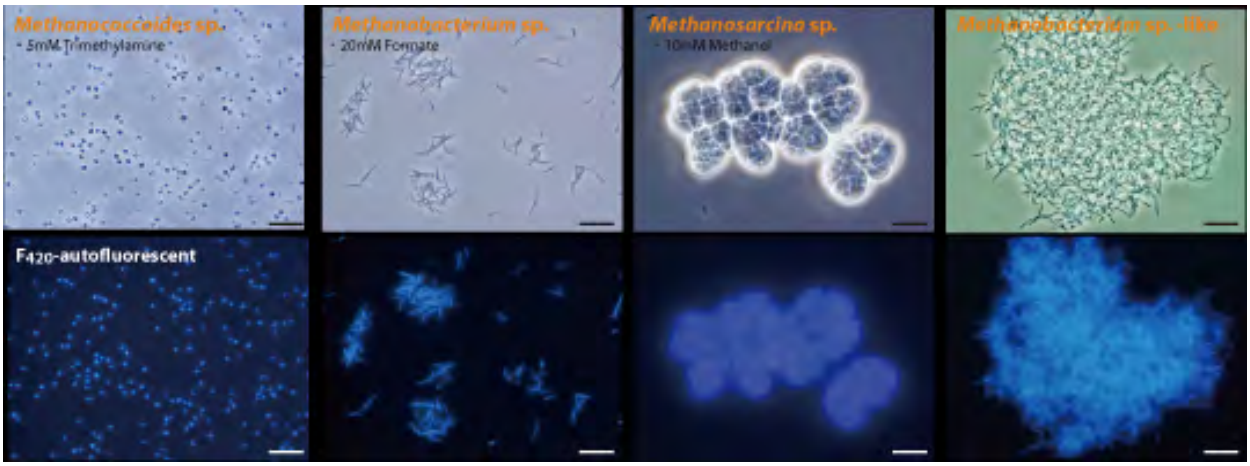

Fig. 8. Methanogenic archaea isolated from sediments at Site C9001 using a flow-through bio-reactor system. The photos show microscopic image of isolated methanogens that produce auto-fluorescence from F430 under UV irradiation as shown in the bottom photos (same microscopic field images) (Imachi et al., in prep.).

Scientific Objectives and Hypotheses

During Expedition 337, extending the riser drilling/coring depth at Site C9001 is planned down to 2,200-2,500 mbsf, where the terrigenous Eocene coalbed (lignite) is situated beneath the overlying marine sedimentary realm (Fig. 4). In addition, using Hybrid-PCS (pressure-coring system) on *Chikyu*, the shallow sedimentary sections down to the maximum depth of 360 mbsf will be retrieved under *in-situ* pressure condition, including methane hydrate-bearing sediments (Fig. 5).

The proposed drilling exploration of the deep hydrocarbon system off Shimokita provides the unique opportunity to examine geobiological and diagenetic processes at interfaces between marine and terrigenous sediment and coal formation in deeply buried strata. No microbial life and its activities have been documented to date at the targeted burial depths and the environment. Expedition 337 will be driven by three overarching testable hypotheses:

1. The deeply buried Eocene coalbed acts as geobiological reactor that releases dissolved organic compounds such as methane, acetate and other substances.
2. The conversion and transport of the coalbed derived organic substances influence microbial and diagenetic processes in the overlying, shallower strata.
3. The subsurface coalbed has the potential to serve as a cap rock for potential future activities of CO_2 sequestration and can support biological conversion of CO_2 into biomass and organic compounds even under high CO_2.

The following operational objectives to be addressed during Expedition 337 will be tied to the above hypotheses and guide our research strategy:

(I) Constraining the impact of a thermally immature coalbed on the diagenetic and microbial processes at great burial depths,
(II) Quantifying the upward fluxes of dissolved organic compounds such as gaseous hydrocarbons and volatile fatty acids out of the coalbed and evaluate their impact on

microbial processes in shallower strata, and
(III) Testing whether distinct active microbial communities inhabit the deeply buried coalbed, the overlying sediments of terrigenous origin and the even shallower marine sediments, and how they respond to high CO_2.

We will address the following set of specific research questions:

- What is the ecological and biogeochemical relevance of deeply buried lignite in the natural hydrocarbon system offshore the Shimokita Peninsula?
- What are the fluxes of both thermogenically and biologically produced methane and other diagenetic products such as organic acids into shallower strata and how important are these for the carbon budget?
- How does the coal diagenesis affect subseafloor microbial biomass, diversity and metabolic activities?
- Does the presence of the low-maturity coalbed stimulate heterotrophic and autotrophic microbial communities?
- What is the natural flux of CO_2 and CH_4 from the coalbed hydrocarbon system, and what is the potential for CO_2 sequestration in the Shimokita system?
- How does excess CO_2 react with minerals and organic matter in the drilled formation, how will this change the physical and chemical characteristics, and how will it affect the microbial communities?
- What is the paleoenvironmental information recorded at Site C9001?
- What is the extent of subseafloor life and the biosphere?

During Expedition 337, we will meet these objectives by: (i) spot-coring marine (Pliocene to Oligocene) and terrestrial (Eocene) sediments, which include unconformity layers as well as coal-tuff-sand layers, (ii) wireline logging of various geophysical and geochemical properties *in-situ*, (iii) sampling of *in-situ* pristine formation fluids using wireline sampling tool and (iv) undertaking extensive microbiological, biogeochemical, geological and geophysical analyses of the cores and borehole-logging data.

The project will expand our knowledge of geobiological and biogeochemical properties in the coalbed hydrocarbon system. Similar coaly environments are widely distributed along the western coast of the Pacific Ocean, and hence our results will be of great societal relevance. Since the effect of high CO_2 concentrations and the associated decrease in pH under conditions of CO_2 sequestration into the deep coal/sand-layers is one of the primary objectives to be addressed, the shore-based laboratory experiments will include quantitative evaluation and modeling of fluid flow and biological systems in the subseafloor environment, including their response to high CO_2. These applied scientific aspects will add an important new component to IODP.

Drilling and Coring Plan

We depart the port of Hachinohe and move to Site C9001 (41°10.5983' N, 142°12.0328' E). After the sea floor survey by ROV, we deploy transponders on the seabed, and then spud in the suspended hole where 20'' casing pipes were previously installed down to 511 mbsf. Corrosion cap is

retrieved, and then blow out preventer (BOP) and riser pipes will be connected to the borehole. We conduct riser drilling down to 1,220 mbsf with spot coring by using rotary core barrel (RCB). The spot coring is planned every 150 m, at the following four depth intervals: 670-679.5 mbsf, 820-829.5 mbsf, 970-979.5 mbsf and 1,120-1,129.5 mbsf (Table 1). Then the first series of wireline logging runs will be performed for the depth interval between 647 and 1,220 mbsf before installing 13-3/8'' casing pipes (see: Logging, Downhole Measurements and *In-situ* Sampling Plan).

From 1,220 mbsf to the target depth of 2,200 mbsf, we continue riser drilling to take cores at the following depths: 1,270-1,279.5, 1,370-1,379.5, 1,470-1,479.5, 1,581.5-1,648, 1,648-1,675, 1,770-1,789, 1,870-1,889, 1,933-1,990, 1,990-2,044, 2,140-2,149.5, and 2,190.5-2,200 mbsf. The coring at 1,648-1,675 and 1,990-2,044 mbsf will utilize Large Diameter Coring (LDC) systems (Baker Hughes INTEQ, Inc.) (Table 1). The LDC systems provide core material of 10 cm (4 inch) in diameter and up to 27 m in length. The LDC system is equipped with either Hydrolift or Jam-Buster system, which improve core recovery particularly in problematic lithologies such as brittle lignite. Core retrieval by LDC system requires pipe trip, not wire line trip, and is contained in an aluminum inner tube. The LDC system will be used for two critical intervals, one corresponding to the Eocene-Oligocene unconformity and the other for the central part of Eocene unit that contains the lignite layers. After reaching the target depth of 2,200 mbsf, we will conduct the second series of wireline logging, including *in-situ* sampling of the formation fluids. The hole will be suspended by cementing without additional casing pipe installations.

After completion of the riser hole, we conduct coring at C9001 by Hybrid-Pressure Coring System (Hybrid-PCS: Aumann & Associates, Inc.). Hybrid-PCS contains a bearing and a nitrogen-charged pressure regulator section, enabling recovery of a 54 mm-diameter and 3.5-m long core in *in-situ* pressure condition. Using the Hybrid-PCS, we collect spot-core samples at several representative depth intervals: 0-3.5 mbsf, 10-13.5 mbsf, 100-103.5 mbsf, and 206.5-231 mbsf (Table 1). During the non-riser Hybrid-PCS operation, formation temperature is measured with APCT-3 at selected depth horizons. Retrieved cores are transferred without pressure loss into an aluminum chamber in the Pressure Core Analysis and Transfer System (PCATS: GeoTek, Ltd., UK). PCATS system enables us to measure p-wave velocity and gamma density, as well as non-destructive 2D and 3D X-ray CT images (Schultheiss et al., 2009).

The currently projected depth intervals for coring are shown in Table T1. We intend to recover sediments from all representative lithologies. At the same time, relatively regular spacing of spot coring is useful to obtain reliable variation of physical properties with depth. Actual coring intervals are subject to change onboard, based on initial results of coring and observation of cuttings and logging data. Analysis of cuttings, cored sediments, log data and seismic integration will be an important task of science party.

Table T1. Projected coring depth intervals for Expedition 337.

Hole	Start depth (mbsf)	End depth (mbsf)	Length (m)	Drilling Method	Coring Method	Expected Sedimentary Age and Lithological Characteristics
A	670.0	679.5	9.5	Riser	RCB	Upper Pliocene hemi-pelagic mud
A	820.0	829.5	9.5	Riser	RCB	Middle Pliocene hemi-

						pelagic mud
A	970.0	979.5	9.5	Riser	RCB	Lower Pliocene hemi-pelagic mud
A	1120.0	1129.5	9.5	Riser	RCB	Pliocene-Miocene unconformity
A	1270.0	1279.5	9.5	Riser	RCB	Miocene-Oligocene hemi-pelagic mud
A	1370.0	1379.5	9.5	Riser	RCB	Oligocene hemi-pelagic mud
A	1470.0	1479.5	9.5	Riser	RCB	Oligocene sandstone
A	1581.5	1648.0	9.5 (x7)	Riser	RCB	Oligocene sandstone
A	1648.0	1675.0	27.0	Riser	LDC	Oligocene-Eocene unconformity
A	1770.0	1789.0	9.5 (x 2)	Riser	RCB	Eocene lacustrine mud
A	1870.0	1889.0	9.5 (x 2)	Riser	RCB	Eocene lacustrine mud
A	1933.0	1990.0	9.5 (x 6)	Riser	RCB	Eocene lignite
A	1990.0	2044.0	27.0(x 2)	Riser	LDC	Eocene lignite
A	2140.0	2149.5	9.5	Riser	RCB	Eocene lacustrine mud
A	2190.5	2200.0	9.5	Riser	RCB	Eocene lacustrine mud
B	0	3.5	3.5	Non-riser	Hybrid-PCS or HPCS	Mudline sample
B	10.0	13.5	3.5	Non-riser	Hybrid-PCS or HPCS	Quaternary sediment
B	100	103.5	3.5	Non-riser	Hybrid-PCS	Quaternary sediment
B	206.5	231.0	3.5 (x7)	Non-riser	Hybrid-PCS	Hydrate-bearing sediment

Logging, Downhole Measurements and *In-situ* Sampling Plan

In order to fill the gap of information between spot coring intervals, the logging program is essential to document geophysical, geochemical, hydrogeological and geobiological properties in the Shimokita coalbed hydrocarbon system. In addition, *in-situ* sampling of formation fluids as well as *in-situ* measurement of pH, hydrocarbon composition and pCO_2 in the formation fluid using logging tools will provide information that is important for the understanding of the coalbed hydrocarbon system.

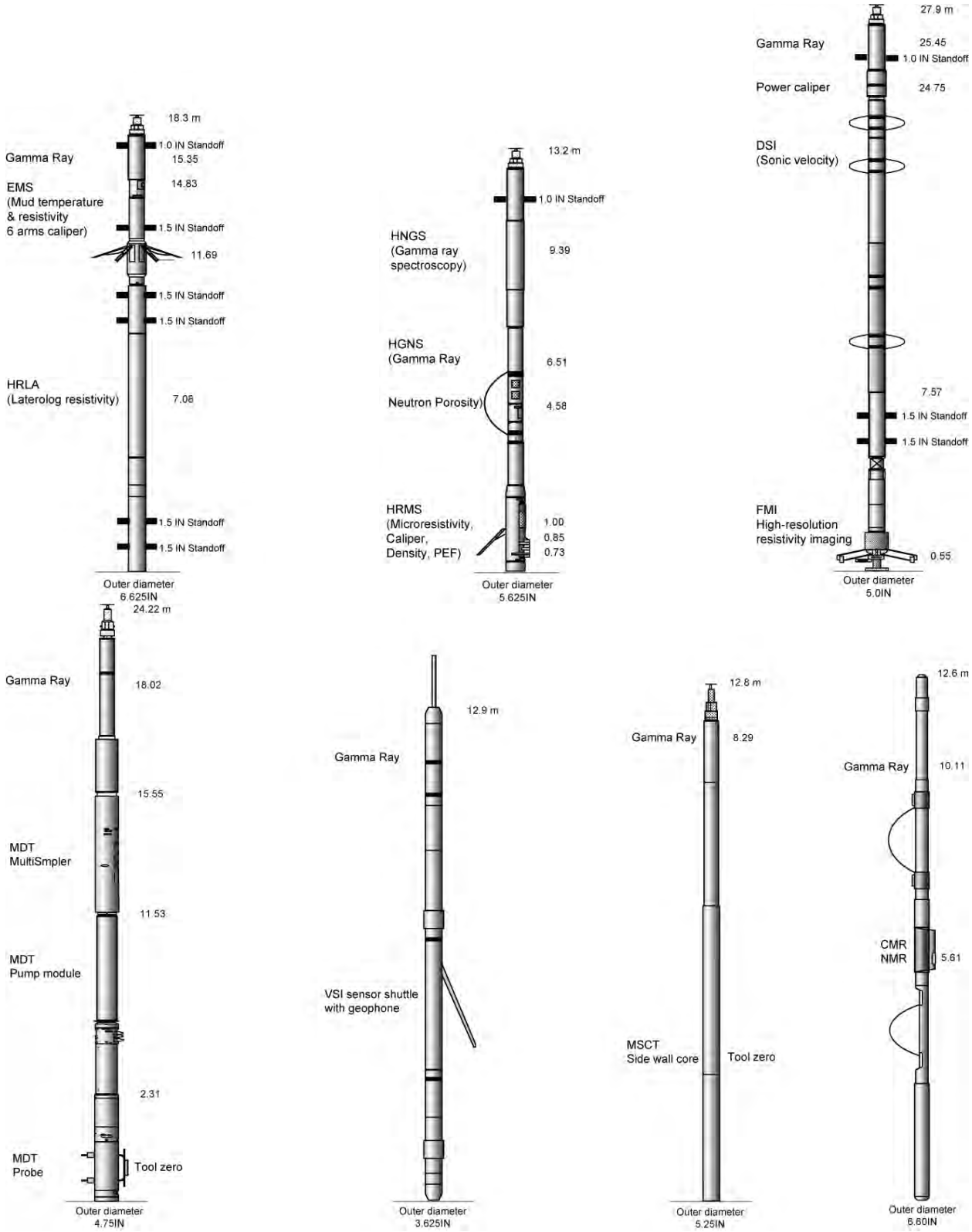

Fig. 9. Sketch of wire line logging tools for the first three runs (top) and the subsequent four runs (bottom).

During Expedition 337, we conduct wireline logging operations at two depth intervals: one

for 647- 1,220 mbsf and the other for 1,220 – 2,200 mbsf. The current logging plan consists of four runs in first shallow interval, and seven runs in the second interval. Tool sketches and configuration figures are shown in Fig. 9.

1. High-Resolution Laterolog Array (HRLA) tool for latero-resistivity,
2. Platform Express (PEX) for density, porosity, 1-arm caliper, micro-resistivity, photo electric factor and Hostile Environment Natural Gamma Ray Sonde (HNGS) for gamma-ray spectroscopy,
3. Fullbore Formation MicroImager (FMI) for electrical borehole image and Dipole Shear Sonic Imager (DSI) for P and S sonic velocity,
4. Versatile Seismic Imager (VSI) for vertical seismic velocity profile by check shot.
5. Combinable Magnetic Resonance (CMR) tool for Nuclear Magnetic Resonance porosity, permeability, pore-seize distribution.
6. Modular Formation Dynamics Tester (MDT) for collecting *in-situ* formation fluid samples using Quicksilver probe and measurement of resistivity, pressure, temperature, hydrocarbon composition (C1 to C6), pH, pCO_2, using *In-situ* Fluid Analyzer (IFA) at the sampling line.
7. Mechanical Sidewall Coring Tool (MSCT) for retrieval of multipe mini-cores from borehole sidewall.

The first two runs provide basic physical properties, such as natural gamma ray, resistivity, density, photoelectric factor and porosity, providing useful data for characterizing high-resolution stratigraphy within the formation. The caliper log allows us to assess the hole conditions and chances of success of subsequent logging runs.

The high-resolution FMI images in the third run provide fine-scale stratigraphy and the best information about the extent of deformation and brecciation. DSI provides the first measurements of *in-situ* formation sonic velocity, allowing generation of synthetic seismograms for detailed seismic log correlations and characterization of the petrophysical properties.

The fourth run is a check shot using VSP to tie the well data to the seismic survey data. Data will be recorded with VSI containing a three-axis geophone.

The subsequent three runs will only be used in the second interval. High-resolution nuclear magnetic resonance (NMR) measurement provides the porosity and the distribution of pore size within the formation. These data are processed to estimate the permeability and pore-throat size, which is essential to evaluate the chance of success and operation plan of the subsequent formation fluid sampling.

MDT using Quicksilver probe, which is an *in-situ* fluid sampling module, will provide formation fluid samples with minimal contamination. IFA measures fluid resistivity, temperature, pressure, pH, pCO_2 and hydrocarbon concentrations in the sampling port line of MDT. Prior to opening the valve for bottle sampling, the real time monitoring of the quality of sampled fluids will be performed with IFA, allowing the best timing for sampling of pristine formation fluids. These *in-situ* fluid measurement and sampling operations will be carried out at intervals of representative lithology, including intervals across the unconformity and coal-bearing (or organic-rich) permeable layers. The actual sampling strategy, however, will depend on borehole conditions, and measurement target depths will be selected after observations of FMI images, EMS calipers and other logs from previous runs.

If time and budget allow, MSCT will be attempted to retrieve mini-core samples (0.92 inch

in diameter) from the scientifically important horizons (e.g., unconformity layers, coalbed, coal-associated sands and breccia) and/or the RCB spot-coring gaps. The sidewall mini-core samples will be used for the study of sedimentological, geochemical and microbiological characteristics. Deployment of MSCT is still uncertain, however, depending on further review of operational constraints, together with scientific priorities.

All logging data are processed onboard to interpret sedimentary facies and to integrate the data with those from core, mud-logging and seismic profiles to construct geological and hydrogeological models. These models will be fundamental for constraining the flux of substrates from the coalbed into shallower strata and the surrounding area.

Details of logging operation are subject to change for operational reasons.

Analytical Research Plan

Core flow for onboard measurements and storage

Despite the multiple coring strategies and disconnected intervals, we will follow IODP standard measurements in onboard laboratory. All sections (1.5 m in length) go through X-ray CT scan and further analysis/sampling strategy will be determined based on the CT observation. Intervals for whole round sampling will be determined at this point for microbiological analysis/sampling and interstitial water extraction. The fluid samples will be subjected to standard analyses of interstitial water chemistry.

Cores will be split into working and archive halves after physical property measurements by multi-sensor core logger (MSCL). Archive halves will be used for visual core descriptions and image/color scan. Working halves will be used for physical property measurements, such as thermal conductivity and shear strength. Discrete samples will be used for measurements of moisture and density, P-wave velocity, and bulk chemistry. Bulk carbon and sulfur analyses, and organic matter maturity measurements by Rock Eval will provide constraints on chemical processes in and around the coal beds.

Cryogenic Magnetometer will be unavailable through the expedition due to current upgrading of the equipment. Considering the sparse coring, we have to omit onboard measurements of remnant magnetism.

After the onboard processing, all the cores will be packed anaerobically in oxygen-impermeable bags filled with N_2 and stored at 4 ^{o}C. The anaerobic storage at 4 ^{o}C will maintain subseafloor microbial activities; hence, these sediments can still be used for a variety of additional post-cruise microbiological and biogeochemical studies.

Cuttings analysis

In addition to core samples, cuttings material recovered from circulating drilling mud are also available for scientific analysis. Due to the sparse coring, we will rely on micropaleontological observation of cuttings for age determination. Lithology observation is also important for decisions regarding the drilling/coring strategy onboard.

Gas monitoring

During the riser-drilling operation, we will monitor the chemical composition (C1/C2+) of mud gas with gas chromatography deployed in a newly constructed mud-gas container lab on *Chikyu*. Circulating drilling mud will be sampled as soon as possible, and the resulting gas sample will be

transferred to the mud-gas container lab through the onboard flow-through pipeline. Carbon isotopic composition of methane ($\delta^{13}C_{CH4}$) will be continuously monitored using an automated wavelength-scanned cavity ring down spectrometer. These mud-gas measurements will fill the gap of coring intervals, and be useful to monitor how biological and non-biological diagenetic processes affect the vertical transition of the coalbed hydrocarbon system as well as the relationship between gaseous components and lithostratigraphy. Mud-gas samples will be also available for more detailed shore-based analysis, such as hydrogen isotopic composition of methane.

High-pressure core

During the non-riser operations using Hybrid-PCS and PCATS system, a 3.5 m-length high-pressure core will be transferred to an aluminum high-pressure chamber with PCATS system in the cooled container. During the transfer operation, p-wave velocity and gamma-density data as well as X-ray CT scan images will be simultaneously obtained by the non-destructive measurement in PCATS. Using the high-pressure chamber system, we will measure *in-situ* chemical composition and concentration of free-hydrocarbon gas.

Sampling strategy

Shipboard and shore-based researchers should refer to the IODP Sample, Data, and Obligations policy posted on the Web at www.iodp.org. This document outlines the policy for distributing IODP samples and data to research scientists, curators, and educators. The document also defines the obligations that sample and data recipients incur. The Sample Allocation Committee (SAC; composed of co-chief scientists, staff scientist, and IODP curator on shore and curatorial representative on board ship) will work with the entire scientific party to formulate a formal expedition-specific sampling plan for shipboard and post-cruise sampling.

Shipboard scientists are expected to submit sample requests (at smcs.iodp.org) 2 months before the beginning of the expedition. Based on sample requests (shore based and shipboard) submitted by this deadline, the SAC will prepare a tentative sampling plan, which will be revised on the ship as dictated by recovery and cruise objectives. The sampling plan will be subject to modification depending upon the actual material recovered and collaborations that may evolve between scientists during the expedition. Modification of the strategy during the expedition must be approved by the co-chief scientists, staff scientist, and curatorial representative on board ship.

The minimum permanent archive will be the standard archive half of each core. All sample frequencies and sizes must be justified on a scientific basis and will depend on core recovery, the full spectrum of other requests, and the cruise objectives. Some redundancy of measurement is unavoidable, but minimizing the duplication of measurements among the shipboard party and identified shore-based collaborators will be a factor in evaluating sample requests.

If some critical intervals are recovered, there may be considerable demand for samples from a limited amount of cored material. These intervals may require special handling, a higher sampling density, reduced sample size, or continuous core sampling by a single investigator. A sampling plan coordinated by the SAC may be required before critical intervals are sampled.

Routine Microbiology Samples (RMS) will be reserved for archiving purpose. A 10-cm WRC is taken at every 10 m of core, and is kept –at -80 ºC. These samples will be available for future microbiological studies onshore.

Contamination Assessment for Riser Coring

For geochemistry and microbiology, determining a sample's degree of contamination with alkaline (~pH 10) mud circulation fluids will be crucial, also because some facultative anaerobic halophilic or halo-tolerant microbes such as *Halomonas* may grow in the circulation mud tank (Masui et al., 2008). Also, the pore waters squeezed from consolidated sedimentary rocks will be highly sensitive to contaminating chemicals. During Expedition 337, we will test the use perfluorocarbon tracers (PFT) for all riser-drilling cores. In the circulation mud tank, PFT concentrations will be kept at 1 ppm (approximately half the concentration of saturation in seawater). During the mud circulation, 1 kg of PFT will be supplemented with 100 m^3 of mud in the tank. The tracer permeation in core sections will be evaluated with a gas chromatograph in the microbiology lab on *Chikyu* according to previously established protocols (Smith et al., 2000; House et al., 2003; Lever et al., 2006).

Subseafloor biomass profiling with multiple methods down to 2,200 mbsf

IODP Expedition 337 will provide an unprecedented opportunity to study deep subseafloor microbial communities inhabiting organic-rich, gassy sediments down to 2,200 mbsf. The targeted depth of maximum penetration is similar to or even extending the previous depth-record of scientific ocean drilling, which is currently at 2,111 mbsf, and held by *JOIDES Resolution* during the Ocean Drilling Program Leg 148, Hole 504B off Costa Rica (Alt et al., 1993). Since the current depth record of the existence of subseafloor life is at 1,626 mbsf at the Newfoundland Margin (Roussel et al., 2008), our study of vertical distribution of microbial biomass will significantly extend our understanding of the extent of subseafloor life and the biosphere on Earth. In addition, the analysis of the distribution and quantity of subseafloor biomass will provide primary information on how microbial populations are sustained by flux of nutrients and electron donors and acceptors, sediment porosity and fluid flow regimes along the unconformity layers and other lithostratigraphic interfaces (e.g., coal-sand interface).

To detect and quantify the biomass of deep biosphere microbial populations, we will use a newly developed computer-based image analysis (Morono et al., 2009). To detach the cells effectively, the sediments will be washed with hydrofluoric acid and the microbial cells will be separated from solid sediment particles using the Nicodenz gradient method (Kallmeyer et al., 2008). Using SYBR-stained cells, the cell detection and enumeration will be performed with an automated slide-leader system equipped with auto-focused fluorescent microscopy (Morono & Inagaki, 2010). We will also compare results using the new high-throughput cell counting technique for geological habitats, based on high-spec flowcytometry (FCM) (Morono & Kallmeyer, personal communication) (Fig. 10). In addition, we will use established protocols involving the analysis of intact polar membrane lipids (e.g., Lipp et al., 2008).

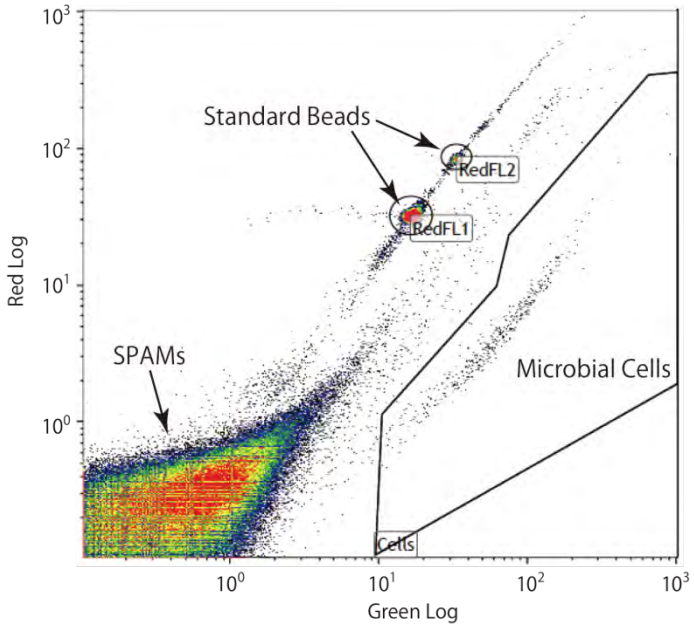

Fig. 10. An image of spectrum separation of SYBR-stained microbial cells from background matrix of SYBR-stainable particulate matter (SYBR-SPAMs) using high-throughput flow cytometry.

Microbial community composition across the marine-terrestrial interface

We will study microbial diversities and community structures using molecular ecological approaches. Most microorganisms are expected to be uncultured heterotrophs, as previously observed at other organic-rich subseafloor sedimentary environments like ODP Sites 1229 and 1230 off Peru (e.g., Parkes et al., 2005; Biddle et al., 2006; Inagaki et al., 2006). For deep-biosphere communities, the relevance of paleoenvironmental conditions during sediment deposition in structuring the microbial community composition, diversity richness and evenness remains unknown. What are the chemical or geophysical constraints on the microbial community structures? How do lithostratigraphic variations play a role for the migration or stratification of microbial communities? To address these fundamental questions related to the diversity and community structure, we will study samples from all recovered lithostratigraphic units corresponding to marine and terrestrial deposits and coal layers.

With regard to the molecular techniques, DNA (and/or RNA) will be extracted from sediment core samples by a newly developed extraction technique that minimizes bias (Morono et al., unpublished data). The cell lysis efficiency will be determined for all samples by checking the number of SYBR-stainable cell particles using an automated fluorescent image analysis (Morono et al., 2008; Morono & Inagaki, 2010). 16S rRNA gene and other functional gene fragments will be amplified with tagged primer sets, sequenced with 454 pyrosequencing and/or other high-throughput sequencing technologies, and then statistically and phylogentically analyzed. In addition, some specific phylotypes of biogeochemically relevant key players (e.g., methanogens, acetogens) will be visualized by FISH-based techniques using specifically designed probes.

Geobiological studies of autotrophic microbes

To understand the potential carbon flow patterns in the coalbed subseafloor microbial ecosystem, understanding of distribution, diversity and functioning of autotrophic microbial

communities (i.e., CO_2-assimilating microbes) is important because of their capability to convert inorganic substrates into organic matter. Autotrophs such as homoacetogens may play an important role by converting CO_2 to acetate and biomass in an artificial CO_2 deposit. Both reactions, CO_2-reduction to methane and to acetate, are likely exergonic in porewater with elevated dissolved H_2 concentrations as a result of lignite diagenesis. Therefore, we will pay special attention to the population and activity of methanogens and other autotrophic communities using multiple cultivation and culture-independent approaches, including an analysis of their sensitivity towards high CO_2 and low pH.

To understand the methanogenic archaeal populations, a key gene for methanogenesis pathway, methyl-coenzyme M reductase (MCR) genes will be phylogenetically characterized and quantified by PCR-based molecular ecological techniques (e.g., Colwell et al., 2008). We also study the coenzyme F430, the specific nickel-containing prosthetic group by multiple structural analyses, such as spectroscopic analysis, high-resolution X-ray structure analysis, and TOF-MS analysis. In addition to these culture-independent analyses onshore, we will cultivate methanogens and other deep subseafloor microbes using a flow-through bioreactor system under the *in-situ* high-pressure and temperature. A variety of methanogens have already been successfully retrieved from the shallow subsurface sediments at the same site. However, we still do not know what kinds of methanogens and associated communities are mainly fueling the significant accumulation of biogenic methane above the coal layer. Using the high-pressure flow-through reactor system, we will also study carbon- and hydrogen-isotopic fractionation of methanogens in the subseafloor sedimentary microbial ecosystem. The expected cultivation-based evidence will contribute to the understanding of the hydrocarbon system and the microbial potential for CO_2 conversion.

Inorganic and organic geochemistry: Understanding the deep carbon cycle

During the *Chikyu* shakedown expedition CK06-06 in 2006, the shallow sedimentary unit above the BSR at 365 mbsf contained methane hydrate in porous ash and sandy layers. Given the moderately low concentrations of organic carbon of 0.8% to 1.8% in the strata, it is conceivable that the methane originates from greater depths, presumably the deeply buried coalbed. Diffusion of biologically produced methane from organic-rich into overlying organic-lean sediments has been demonstrated at other deep drilling sites, e.g., from deeply buried Cretaceous black shales drilled during ODP Leg 207 at Demerara Rise (Arndt et al., 2006). Using cored materials, we will conduct detailed analysis of numerous geochemical parameters that will enable us to quantify the fluxes of various compounds from and into the Eocene coalbed. Concentrations of major anions and cations (e.g., sulfate, chloride, alkalinity, sulfide, phosphate; ammonium, magnesium, potassium, calcium) in porewater samples and formation fluids will be determined. We will determine concentrations and stable carbon isotopic compositions of various carbon-bearing compounds: i.e., CH_4 and C_{2+} hydrocarbons, volatile fatty acids, DOC, and DIC (e.g., Heuer et al., 2009: Lever et al., 2010). We will determine δD values of CH_4 in order to distinguish between different pathways of methanogenesis (cf. Whiticar, 1999). The dissolved organic matter in the pore and formation water and its structural link to lignite-derived organic compounds will be studied by FT-ICR-MS (e.g., Schmidt et al., 2009). We will seek to apply new assays based on solid phase micro-extraction for the quantification and isotopic analysis of methylotrophic substrates such as methylamines and methanol in pore fluids. This set of analyses will provide information pertinent to the geobiological carbon cycling and will enable us to model fluxes of carbon-bearing compounds in and out of the

coalbed (e.g., Sivan et al., 2007; Wang et al., 2008). We will determine concentrations and stable isotopic compositions of organic carbon, carbonate, nitrogen, and sulfur, and hydrogen indices by Rock Eval Pyrolysis to further characterize the diagenetic setting and broadly distinguish sources of organic material. Some of these techniques will be applied to selected, cleaned core cuttings in order to supplement geochemical data with continuous information. In addition, we will measure $^{129}I/^{127}I$ ratios of pore waters to examine the age distribution of pore water enriched in iodine and methane in gas-rich strata (e.g., Tomaru et al., 2009).

Biogeochemical and geobiological experiments: Activities and fluxes

We will use large-volume samples of live sediments that are taken in regular intervals of one per recovered core (i.e., WRC) to conduct laboratory incubation to monitor the production potential for methane and methanogenic substrates such as methyl-compounds, acetate, H_2. To better assess fluxes of compounds in organic-rich strata and in the adjacent sediment horizons, we will quantify microbial respiration processes by high sensitivity methods such as radiotracer turnover based on ^{14}C-, ^{35}S- and deuterium-labeled compounds. These *ex-situ* experiments will inform us on the potential activity of the coalbed microbial communities and provide information on the reactivity of the lignite. These experiments will be anaerobically conducted at near *in-situ* temperature in the newly developed radioisotope container lab on *Chikyu*. Rate measurements directly related to methanogenesis are the priority, such as acetate turnover, methanol turnover, methane turnover, and CO_2 turnover. In addition, bulk community growth can be studied by thymidine incorporation. As microbial communities in these experiments, we will use both the natural indigenous microbial population and microbial inoculates that have been tested in coal-to-methane degradation (e.g., Jones EJP et al., 2008; Jones DM et al., 2008; Orem et al., 2010). The inocula also include mesophilic to thermophilic methanogens isolated from subseafloor sediments in shallow zone of the same drilling site (C9001) and from high-CO_2 hydrothermal fluids in the Okinawa Trough hydrothermal fields. In selected experiments, we will use stable isotope-labeled substrates in order to establish reactant-product relationships (e.g., ^{13}C-methanol, ^{13}C-DIC, etc.) and quantify the relative importance of the various pathways metabolizing C_1-compounds (e.g., Wegener et al., 2008). The rate of ^{13}C-labeled substrate incorporations will be determined at single-cell levels using a combined FISH-NanoSIMS approach (Musat et al., 2008) as well as on the basis of microbial biomarker analysis (e.g., Wegener et al., 2008). Overall, these experiments will provide information crucial to the assessment of the lignite's potential to generate methane and other dissolved organic species and will inform the design of experiments under *in-situ* pressure and temperature.

Shore-based study of the offshore CO_2 sequestration potentials: Does deeply buried coalbed act as "Subsea Forest"?

One of the great concerns regarding geological CO_2 storage is the behavior of CO_2 and its impact on ecological balance of carbon cycling. To store substantial quantities of CO_2 in the deep underground or marine subsurface, the captured CO_2 is condensed as liquid. At the high pressure and high temperature in the deep subsurface, the CO_2 will be present in supercritical state. Where liquid or supercritical CO_2 is in contact with a water phase, the CO_2 levels will be in equilibrium with the liquid CO_2, thus be saturated. The pH of pore water or solvent water for CO_2 sequestration will be significantly decreased; hence, the mineral trap via carbonation is extremely low. A diffusion

of liquid CO_2 through the surrounding pore spaces is expected. However, the dense liquid or supercritical CO_2 is hydrophobic and thus would hardly mix with pore water. These physical and chemical characteristics of CO_2 suggest that once liquid and/or supercritical CO_2 is injected in deep subsurface repositories, CO_2 may be retained and these unusual artificial environments may remain stable over geologic time scales. Biological conversion of the injected CO_2 in the subsurface to organic compounds such as methane will depend on how microorganisms will respond to the chemical perturbation and on the intrinsic reducing power of the subsurface environment. In fact, C1-metabolizing microbial life has been observed in the natural deep-sea CO_2-seep and hydrothermal environments (Inagaki et al., 2006b).

What are the drivers in microbial succession in the deep subseafloor biosphere? Previous deep-biosphere studies using ^{14}C-tracer demonstrated that potential rates of CO_2-reducing methanogenesis in typical coastal organic-rich marine sediments are a few tens pmol cm^{-3} day^{-1} (as reviewed by Parkes et al., 2000). In contrast, the activity of batch-cultured thermophilic methanogens is approximately 0.1 to 1 mmol cm^{-3} day^{-1}, that is, roughly eight to nine orders of magnitude higher than subseafloor methanogenesis activity. As a consequence of hypothetical carbon storage, CO_2 concentration could approach 1 mol l^{-1}, the conversion of CO_2 to current ambient levels by the indigenous subseafloor microbial ecosystem would take over hundreds of millions years. However, if there are substantial sources of energy and reducing power such as H_2 or acetate generated via the diagenesis of organic matter at elevated temperatures (Parkes et al., 2007), the CO_2 turnover time may be significantly reduced based on the potential activity of methanogens. We suggest that the potentially active and abundant microbial communities associated with the deeply buried coalbeds –the so called "Subsea Forest"– constitute a highly interesting target for testing CO_2 and pH effects on subsurface life, including effects on C_1-metabolisms, heterotrophic consumption, lipid and DNA formation, and carbon assimilation.

To produce such energy and nutrient sources for microbes, the coal needs to be relatively immature because then it still contains not only hydrogen but also N- and P-bearing compounds, and the porosity of lignite is generally higher than that of more mature graphite coal. Indeed, microbiological studies of terrestrial coal environments revealed the presence of hydrogenotrophic methanogens (Shimizu et al., 2007; Krüger et al., 2008: Strapoc et al., 2008). The coals in the Shimokita gas field are mostly composed of lignite, and the coals are intercalated with porous sandy layers like lens-structure (Osawa et al., 2002). Along with the migrating CO_2, it is possible that dissolved organics or reduced chemical compounds may be advected by the CO_2 conveyor, and fuel heterotrophic respiration (Onstott, 2005). Especially, energy sources co-migrating with liquid CO_2 such as sulfide, methane or H_2 may be oxidized by the subsurface microbiota driving autotrophic growth, or – if physiological functions are repressed by low pH – will not be utilized and transported further. Using high-pressure reactors, sediments samples from Expedition 337 will be incubated under high CO_2 and compared to *in-situ* conditions, to assess whether the communities can adapt to high CO_2 and, if so, over which time scale.

Geophysical implications for CO_2 sequestration potentials

During the proposed hydrocarbon expedition, a variety of logging and experimental data will be obtained from the borehole and drilled cores. In particular, the high-pressure immersion experiments using representative core materials and CO_2-rich fluids will provide critical information for simulating the behavior of CO_2 in the deep subseafloor environment. As the significant

geophysical and sedimentologial parameters, permeability, porosity and capacity of CO_2-storage will be experimentally determined using fluid-flow reaction chambers *ex-situ* and under varied temperature and pressure conditions. During and after the incubation, we will evaluate how the CO_2 fluid-rock reaction changes mineral compositions and physical properties of sediments. Using a supercomputer device (e.g., Earth Simulator at JAMSTEC), the analysis of these *in-situ* and *ex-situ* data including the seismic data set will constitute regional 3-dimensional models of CO_2 dispersal with time, migration behaviors and environmental changes (e.g., porosity, pH and pCO_2). The modeling will include rates of biological CO_2 turnover to CH_4 or other hydrocarbons, representing the feedback velocity of CO_2-disposal and the enhanced gas production rates. Also, these computational simulations will provide significant information to plan a scientifically sound set of experiments for the large-scale active experimentation in the future, and will contribute to the preparation of large-scale carbon storage in similar environmental settings around the western coast of Pacific Ocean.

Science Party

To complete the onboard sampling, analyses and logging as described above, Expedition 337 will invite a shipboard scientific party that consists of 25-26 scientists, including the two co-chiefs scientists, sedimentologists, organic and inorganic geochemists, (geo-)microbiologists, physical property specialists, micropaleontologists, and structural geologists. In addition to typical requirements in each of these specialties, particular needs in Expedition 337 include, but are not limited to, the following specific expertise:

- Petroleum Geologists and Sedimentologists with strong interest in biogeochemical diagenetic processes on the deeply buried coalbed and/or shore-based CO_2 sequestration into subseafloor hydrocarbon system.
- Biogeochemists and geochemists with experience/background in porewater analysis of biologically relevant chemical species, in rate measurements using radioactive tracers, in hydrogen and hydrocarbon gas analysis, in mud gas monitoring including isotope analysis, in geochemical modeling of transport and reaction of dissolved constituents, and in quantitative and compositional analysis of dissolved organic matter.
- Microbial Ecologists and Molecular Biologists with experience in onboard cell enumeration using flow cytometry and/or image-based microscopic analysis, in onboard anaerobic and aseptic sampling of sediment or rock, in shore-based anaerobic cultivation techniques using high-pressure chamber and/or flow-through reactor systems, in onboard contamination assessment using tracers such as fluorescent micro-beads and/or perfluorocarbon (PFT), in DNA/RNA-based functional gene analyses, in RNA-based molecular analyses of community structure and/or gene expression, and/or in single cell biology and (meta)genomics.
- Physical property specialists with experience/background in measurement of porosity, permeability and determination of fluid migration in cored materials, sidewall mini-cores and wireline logging data, and/or in reactions between liquid and supercritical CO_2-containing fluids and sedimentary rock/minerals.
- Biostratigraphers with experience in micro- and nanno-fossil age determination in the Northeastern Pacific and terrestrial/coastal deposits, and/or in isotopic analyses using beryllium, rhenium-osmium, and iodine for depositional and fluid age.
- Logging specialists with experience in *in-situ* geophysical and geochemical measurements, in borehole sampling of formation fluids and sidewall mini-cores, and/or in handling and data

processing of multiple logging tools as planned in Expedition 337.

Operational Risk

Hydrocarbons

The drilling site is located near commercial gas production wells, but there was no evidence of over-pressure at the offset well. The previous expedition showed that C1/C2 is > 1000 without detection of >C3 gas in the piston cores to 350 mbsf. The site is positioned within a basement syncline in an area where shallow hazards were considered minimal. Even if hydrocarbons would be encountered, mud weights can be increased to control possible flow from the formation during riser drilling.

Weather

Expedition 337 has been scheduled to take place during spring, when no typhoon or seasonal severe weather is expected. The drilling site is free from strong ocean current. In the case of extreme weather, we would evacuate after de-connection of BOP. Re-entry would be possible.

Contingency and alternate plan

Unforeseen circumstances could result in insufficient time being available to complete the entire operations plan. Examples include collapse of a borehole because of difficult formation conditions (unstable sands), hazardous weather, and hardware failures. In anticipation of challenging and fluctuating environmental conditions, we have included 8 days of contingency for the entire expedition in the operations plan and time estimate. In case further delay in operation takes place, we will adjust the time by reducing the number of spot cores.

References

Alt JC, Kinoshita H, Stokking LB, et al. (1993) *Proceedings of the Ocean Drilling Program, Initial Report* **148**: College Station, TX (Ocean Drillign Program).

Aoike K (ed.) (2007) CK06-06 *D/V Chikyu* shakedown cruise offshore Shimokita. Laboratory Operation Report, CDEX-JAMSTEC, pp. 40-43.

Aoike K, Nishi H, Sakamoto T, Iijima K, Tsuchiya M, Taira A, Kuramoto S, Masago H, Shimokita Core Research Group (2010) Paleoceanographic history of offshore Shimokita Peninsula for the past 800,000 years based on primary analyses on cores recovered by *D/V Chikyu* during the shakedown cruises. *Fossils* **87:** 65-81. (In Japanese with English abstract and figures)

Arndt S, Brumsack H, Wirtz KW (2006) Cretaceous black shales as active bioreactors: A biogeochemical model for the deep biosphere encountered during ODP Leg 207 (Demerara Rise). *Geochimica et Cosmochimica Acta* **70:** 408–425.

Biddle JF, Lipp JS, Lever MA, Lloyd KG, Sørensen K, Anderson R, Fredricks HF, Elvert M, Kelly TJ, Schrag DP, Sogin ML, Brenchley JE, Teske A, House CH, Hinrichs K-U (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 3846-3851.

Brown CJ, Coates JD, Schoonen MAA (1999) Localized sulfate-reducing zones in a coastal plain aquifer. *Ground Water* **37:** 505–516.

Cowen JP, Giovannoni SJ, Kenig F, Johnson HP, Butterfield D, Rappé MS, Hutnak M, Lam P (2003) Fluids from aging ocean crust that support microbial life. *Science* **299:** 120-123.

Colwell FS, Boyd S, Delwiche ME, Reed DW, Phelps TJ, Newby DT (2008) Estimates of biogenic methane production rates in deep marine sediments at Hydrate Ridge, Cascadia margin. *Applied and Environmental Microbiology* **74:** 3444-3452.

Detmers J, Schulte U, Strauss H, Kuever J (2001) Sulfate reduction at a lignite seam: Microbial abundance and activity. *Microbial Ecology* **42:** 238–247.

D'Hondt S, Rutherford S, Spivack AJ (2002) Metabolic activity of subsurface life in deep-sea sediments. *Science*, **295:** 2067-2070.

D'Hondt S, Jørgensen BB, Miller DJ, Batzke A, Blake R, Cragg BA, Cypionka H, Dickens GR, Ferdelman T, Hinrichs K-U, Holm NG, Mitterer R, Spivack A, Wang G, Bekins B, Engelen B, Ford K, Gettemy G, Rutherford SD, Sass H, Skilbeck CG, Aiello IW, Guerin G, House C, Inagaki F, Meister P, Naehr T, Niitsuma S, Parkes RJ, Schippers A, Smith DC, Teske A, Wiegel J, Padilla CN, Acosta JLS (2004) Distributions of microbial activities in deep subseafloor sediments. *Science,* **306:** 2116-2121.

D'Hondt S, Spivack AJ, Pockalny R, Ferdelman TG, Fischer JP, Kallmeyer J, Abrams LJ, Smith DC, Graham D, Hasiuk F, Schrum H, Stancin AM (2009) Subseafloor sedimentary life in the South Pacific Gyre. *Proceedings of the National Academy of Sciences of the United States of America* **106:** 11651-11656.

Domitsu H, Nishi H, Uchida J, Oda M, Ogane K, Taira A, Aoike K, Shimokita Microfossil Research Group (2010) Age model of core sediments taken by D/V Chikyu during the shakedown cruises off Shimokita Paninsula. *Fossils* **87:** 47-64. (In Japanese with English abstract and figures)

Dooley JJ, *et al.* (2006) "Carbon dioxide capture and storage", *In*: Technology Report, Global Energy Technology Strategy Program, Battelle Memorial Institute, College Park, MD.

Engelen B, Ziegelmüller K, Wolf L, Köpke B, Gittel A, Cypionka H, Treude T, Nakagawa S, Inagaki F, Lever MA, Steinsbu BO (2008) Fluids from the oceanic crust support microbial activities within the deep biosphere. *Geomicrobiolgy Journal* **25:** 56-66.

Fry J, Horsfield B, Sykes R, Cragg B, Heywood C, Kim GT, Mangelsdorf K, Mildenhall DC, Rinna J, Vieth A, Zink K, Sass H, Weightman A, Parkes RJ (2009) Prokaryotic populations and activities in an interbedded coal deposit, including a previously deeply buried section (1.6-2.3 km) above 150 Ma basement rock. *Geomicrobiology Journal* **26:** 163-178.

Heuer VB, Pohlman JW, Torres ME, Elvert M, Hinrichs K-U (2009) The stable carbon isotope biogeochemistry of acetate and other dissolved carbon species in deep subseafloor sediments at the northern Cascadia Margin. *Geochimica et Cosmochimica Acta* **73:** 3323-3336.

Hinrichs K-U, Hayes JM, Bach W, Spivack AJ, Hmelo LR, Holm NG, Johnson CG, Sylva SP (2006) Biological formation of ethane and propane in the deep marine subsurface. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 14684-14689.

House CH, Cragg BA, Teske A, ODP Leg 201 Scientific Party (2003) Drilling contamination tests during ODP Leg 201 using chemical and particulate tracers. *Proceedings of the Ocean Drilling Program Initial Report* **201:** 1–18.

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, Nealson KH, Horikoshi K (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Applied and Environmental Microbiology* **69:** 7224-7235.

Inagaki F, Nunoura T, Nakagawa S, Teske A, Lever M, Lauer A, Suzuki M, Takai K, Delwiche M, Colwell FS, Nealson KH, Horikoshi K, D'Hondt S, Jørgensen BB (2006a) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 2815-2820.

Inagaki F, Kuypers MMM, Tsunogai U, Ishibashi J, Nakamura K, Treude T, Ohkubo S, Nakaseama M, Gena K, Chiba H, Hirayama H, Nunoura T, Takai K, Jørgensen BB, Horikoshi K, Boetius A (2006b) Microbial community in a sediment-hosted CO_2 lake of the southern Okinawa Trough hydrothermal system. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 14164-14169.

Inagaki F, Nakagawa S (2008) Spatial distribution of the subseafloor life: diversity and biogeography. *In* Dilek Y (ed.) Links Between Geological Processes, Microbial Activities & Evolution of Life, Springer Science+Business Media B.V., 135-158.

Inagaki F (2010) Deep subseafloor microbial communities. *In*: Encyclopedia of Life Sciences (ELS). John Wilely & Sons, Ltd., Chichester, UK.

Japan Natural Gas Association and Japan Offshore Petroleum Development Association (eds.) (1992) Oil and gas resources in Japan (revised edition). 520pp.

Jones EJP, Voytek MA, Warwick PD, Corum MD, Cohn A, Bunnell JE, Clark AC, Orem WH (2008) Evaluating coal for biogenic methane generation potential using a bioassay to assess coal bioavailability. *International Journal of Coal Geology* **76:** 138-150.

Jones DM, Head IM, Gray ND, Adams JJ, Rowan AK, Aitken CM, Bennett B, Huang H, Brown A, Bowler BF, Oldenburg T, Erdmann M, Larter SR (2008) Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. *Nature* **451:** 176-180.

Kallmeyer J, Smith DC, Spivack AJ, D'Hondt S (2008) New cell extraction procedure applied to deep subsurface sediments. *Limnology and Oceanography: Methods* **6:** 236-245.

Kobayashi T, Koide O, Mori K, Shimamura S, Matsuura T, Miura T, Takaki Y, Morono Y, Nunoura T, Imachi H, Inagaki F, Takai K, Horikoshi K (2008) Phylogenetic and enzymatic diversity of deep subseafloor aerobic microorganisms in organics- and methane-rich sediments off Shimokita Peninsula. *Extremophiles* **12:** 519-527.

Krüger M, Beckmann S, Engelen B, Thielemann T, Cramer B, Schippers A, Cypionka H (2008) Microbial methane formation from hard coal and timber in an abandoned coal mine. *Geomicrobiology Journal* **25:** 315–321.

Krumholz LR, McKinley JP, Ulrich FA, Suflita JM (1997) Confined subsurface microbial communities in Cretaceous rock. *Nature*

386: 64–66.

Lever MA, Alperin M, Engelen B, Inagaki F, Nakagawa S, Steinsbu BO, Teske A, IODP Expedition 301 Scientists (2006) Trends in basalt and sediment core contamination during IODP Expedition 301. *Geomicrobiology Journal* **23:** 517-530.

Lever MA, Heuer VB, Morono Y, Masui N, Schmidt F, Alperin MJ, Inagaki F, Hinrichs K-U, Teske A (2010) Acetogenesis in deep subseafloor sediments of the Juan de Fuca ridge flank: A synthesis of geochemical, thermodynamic, and gene-based evidence. *Geomicrobiology Journal* **27:** 183-211.

Lipp JS, Morono Y, Inagaki F, Hinrichs K-U (2008) Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature* **454:** 991-994.

Masui N, Morono Y, Inagaki F (2008) Microbiological assessment of cieculation mud fluids during the first operation of riser drilling by the deep-earth research vessel *Chikyu*. *Geomicrobiology Journal* **25:** 274-282.

Morono Y, Terada T, Masui N, Inagaki F (2009) Discriminative detection and enumeration of microbial life in marine subsurface sediments. *The ISME Journal* **3:** 503-511.

Morono Y, Inagaki F (2010) Automatic slide-loader fluorescent microscope for discriminative enumeration of subseafloor life. *Scientific Drilling* **9:** 32-36.

Musat N, Halm H, Winterholler B, Hoppe P, Peduzzi S, Hillion F, Horreard F, Amann R, Jørgensen BB, Kuypers MMM (2008) A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **105:** 17861-17866.

Nakagawa S, Inagaki F, Suzuki Y, Steinsbu BO, Lever MA, Takai K, Engelen B, Sako Y, Wheat CG, Horikoshi K, Integrated Ocean Drilling Program Expedition 301 Scientists (2006) Microbial community in black rust exposed to hot ridge flank crustal fluids. *Applied and Environmental Microbiology* **72:** 6789-6799.

Onstott TC (2005) Impact of CO2 injections on deep subsurface microbial ecosystems and potential ramifications for the surface biosphere. *In*: Thomas DC and Benson SM (eds.) "The CO_2 Capture and Storage (CCS)", Vol. 2, pp. 1207-1239, Elsevier Ltd.

Orem WH, Voytek MA, Jones EJ, Lerch HE, Bates AL, Corum MD, Warwick PD, Clark AC (2010) Organic intermediates in the anaerobic biodegradation of coal to methane under laboratory conditions. *Organic Geochemistry* **41:** 997-1000.

Osawa M, Nakanishi S, Tanahashi M, Oda H (2002) Structure, tectonic evolution and gas exploration potential of offshore Sanriku and Hidaka provinces, Pacific Ocean, off northern Honshu and Hokkaido, Japan. *Journal of the Japanese Association for Petroleum Technology* **67:** 37-51. (In Japanese with English abstract and figures)

Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, Fry JC, Weightman AJ, Harvey SM (1994) Deep bacterial biosphere in Pacific Ocean sediments. *Nature* **371:** 410-413.

Parkes RJ, Cragg BA, Wellsbury P (2000) Recent studies on bacterial populations and processes in subseafloor sediments: A review. *Hydrogeology Journal* **8:** 11-28.

Parkes RJ, Webster G, Cragg BA, Weightman AJ, Newberry CJ, Ferdelman TG, Kallmeyer J, Jørgensen BB, Aiello IW and Fry JC (2005) Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* **436:** 390-394.

Parkes RJ, Wellsbury P, Mather ID, Cobb SJ, Cragg BA, Hornibrook Ed RC, Horsfield B (2007) Temperature activation of organic matter and minerals during burial has the potential to sustain the deep biosphere over geologic timescales. *Organic Geochemistry* **38:** 845-852.

Roussel EG, Bonavita MA, Querellou J, Cragg BA, Webster G, Prieur D, Parkes RJ (2008) Extending the sub-sea-floor biosphere. *Science,* **320:**1046.

Seno T, Sakurai T, Stein S (1996) Can the Okhotsk plate be discriminated from the North American Plate? *Journal of Geophysical Research* **101:** 11305-11315.

Schultheiss P., Holland M, Humphrey G (2009) Wireline coring and analysis under pressure: Recent use and future developments of the HYACINTH system. *Scientific Drilling* **7**: 44-50.

Schrag DP (2009) Storage of carbon dioxide in offshore sediments. *Science* **325:** 1658-1659.

Shimizu S, Akiyama M, Naganuma T, Fujioka M, Nako M, Ishijima Y (2007) Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. *Geobiology* **5:** 423–433.

Schmidt F, Elvert M, Koch B, Witt M, Hinrichs K-U (2009) Molecular characterization of dissolved organic matter in pore water in continental shelf sediments. *Geochimica et Cosmochimica Acta* **73:** 3337-3358.

Sivan O, Schrag DP, Murray RW (2007) Rates of methanogenesis and methanotrophy in deep-sea sediments. *Geobiology* **5:** 141-151.

Smith DC, Spivack AJ, Fisk MR, Haveman SA, Staudigel H (2000) Tracer- based estimates of drilling-induced microbial contamination of deep sea crust. *Geomicrobiology Journal* **17:** 207–219.

Sørensen KB, Teske A (2006) Stratified communities of active Archaea in deep marine subsurface sediments. *Applied and Environmental Microbiology* **72:** 4596-4603.

Strapoc D, Picardal FW, Turich C, Schaperdoth I, Macalady JL, Lipp JS, Lin YS, Ertefai TF, Schubotz F, Hinrichs KU, Mastalerz M, Schimmelmann A (2008) Methane-producing microbial community in a coal bed of the Illinois basin. *Applied and Environmental*

Microbiology **74:** 2424-2432.

Taira A, Curewitz D (eds.) (2005) Shimokita area site survey: Northern Japan Trench seismic survey, offshore northern Honshu, Japan. *CDEX Technical Report* **2:** 1-155, ISSN 1880-0875, JAMSTEC-CDEX, Yokohama.

Tomaru H, Fehn U, Lu Z, Takeuchi R, Inagaki F, Imachi H, Kotani R, Matsumoto R, Aoike K (2009) Dating of dissolved iodine in pore waters from the gas hydrate occurrence offshore Shimokita Peninsula, Japan: ^{129}I results from *D/V Chikyu* shakedown cruise. *Resource Geology* **59:** 359-373.

Wang G, Spivack AJ, Rutherford S, Manor U, D'Hondt S (2008) Quantification of co-occurring reaction rates in deep subseaflor sediments. *Geochimica et Cosmochimica Acta* **72:** 3479-3448.

Wegener G, Niemann H, Elvert M, Hinrichs K-U, Boetius A (2008) Assimilation of methane and inorganic carbon by microbial communities mediating the anaerobic oxidation of methane. *Environmental Microbiology* **10:** 2287–2298.

Whiticar MJ (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology* **161:** 291–314.

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences of the United States of America* **95:** 6578-6583.

Site C9001

Priority:	Primary
Position:	41º10.5983' N, 142º12.0328' E
Water depth (m):	1180 m
Target drilling depth (mbsf):	2200 mbsf
Approved maximum penetration (mbsf):	2500 mbsf
Survey coverage (track map; seismic profile):	Primary Lines: ODSRW03H-81~87 Crossing Lines: ODSRW03H-B1~B7
Objective:	Exploration of coalbed-hydrocarbon system and deep biosphere
Drilling program:	Non-riser: 0-365 mbsf, Riser: 650-2200 mbsf
Logging program:	1. High-Resolution Laterolog Array (HRLA) 2. Platform Express (PEX) and Hostile Environment Natural Gamma Ray Sonde (HNGS) 3. Fullbore Formation MicroImager (FMI) and Dipole Shear Sonic Imager (DSI) 4. Versatile Seismic Imager (VSI) for check shot 5. Combinable Magnetic Resonance (CMR) 6. Modular Formation Dynamics Tester (MDT) using Quicksilver probe and In-situ Fluid Analyzer (IFA) 7. Mechanical Sidewall Coring Tool (MSCT) 650-1220 mbsf: #1-4, 1220-2200 mbsf: #1-7
Nature of rock anticipated:	hemi-pelagic silty clay, congromerate, lacustrine sandstone, mudstone, lignite coal