Subsurface Life Task Force Report to IODP-MI

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The community-wide IODP/JOI Workshop on *Exploring Subsurface Life with the Integrated Ocean Drilling Program* brought together 90 international scientists to (chaired by S. D'Hondt and F. Inagaki, October 2006). A general outcome of the workshop was consensus that the microbiological community needs to take advantage of the full range of IODP expedition possibilities to meet the multiple challenges of (1) describing deep subsurface microbial diversity, (2) accurately constraining microbial biomass and activity, and (3) mapping habitable space within the subseafloor ocean (D'Hondt et al., 2007).

To provide an implementation plan in support of that consensus, an IODP Task Force was created to hold a single meeting. The meeting was held September 17-19, 2007, at the IODP-MI office in Washington, D.C. This report constitutes that implementation plan. It defines (1) generic science strategies for study of subseafloor life and habitability, (2) recommendations for standard measurements and legacy samples, (3) recommended protocols for implementing standard measurements and legacy sampling, and (4) recommended requirements for microbiological study of IODP materials. It also recommends concrete steps for encouraging subsurface life studies on IODP expeditions, expeditionary data and legacy samples.

These strategies and recommendations provide specific guidelines for meeting many of the scientific and technological objectives identified by the community-wide workshop. They also define a framework for initial and basic IODP progress on study of subseafloor life within the time frame of 2008 - 2013.

The sample and measurement recommendations build on the Workshop white paper and the 2003 STP Microbiology Working Group Report. The discussions of standard measurements and legacy samples include recommendations on: (1) circumstances or categories of expeditions for which those samples and measurements should be taken. Where appropriate, these discussions also include a strategy for consistently getting the measurements done.

If published as a task force report, the task force recommendations for general sampling and generic strategies may help to guide the broader community in proposal writing.

To undertake this project, IODP-MI paid travel and meeting expenses for 9 people to participate. The task force included multiple representatives from Japan, Europe and the United States. To maximize the depth of and appropriateness of the recommendations, it included scientists who are active in the international drilling community and scientists

who are deeply versed in appropriate microbiological/biogeochemical techniques and study areas but not yet active in the drilling community. An ICDP observer also participated in the meeting.

Generic Science Strategies

The generic science strategies include guidelines for strategies (site location, samples and sample treatment) specific to major scientific themes and objectives identified by the workshop. These generic strategies include scientific objectives that may be best met with

(1) Standard measurements on IODP expeditions,
(2) A legacy sampling program,
(3) Targeted addition of subsurface life studies to IODP expeditions, and
(4) Expeditions specifically dedicated to study of subseafloor life.

Scientific priorities for all categories of objectives are rooted in the 2006 Workshop report. However, the Task Force provides a practical focus for scientific priorities by suggesting implementation priorities.

The case for new biosphere-targeted standard measurements

IODP standard measurements focused on subseafloor life include contamination tests, cell counts on fixed samples, and a few standard measurements of interstitial water chemistry (nutrients, alkalinity, sulfate). Other minimum and standard measurements are relevant because they help to constrain the habitability of subseafloor environments. These include physical properties (discrete-sample density and porosity), lithology (carbonate content), and chemical analyses relevant to redox habitability and electron donors (bulk carbon-hydrogen-nitrogen-sulfur analyses and natural gamma logs).

For sediments, the present standard measurements allow scientists to make very modest progress in mapping three-dimensional patterns of subseafloor habitability, particularly physical and chemical habitability, and subseafloor respiration. However, the standard IODP measurements will not significantly advance understanding of subseafloor life unless they are expanded and paired to a comprehensive legacy sampling program. Even this will not fully constrain subseafloor distributions of microbial communities and habitability because some important subseafloor sedimentary environments (such as regions distant from shore) will not be drilled by IODP unless they are deliberately targeted for study of subseafloor life.

Several key biogeochemical variables are not addressed by standard measurements. These include concentrations of dissolved electron donors (microbial "foods" such as hydrogen, short-chain fatty acids, sulfide, and ammonium) and of electron acceptors such as nitrate and oxygen. Although it is the principal product of organic-fueled respiration, dissolved inorganic carbon (DIC) is not among the standard analytes. The standard measurements of pH do not accurately represent *in situ* conditions. The formation factor (ratio of saturated sediment resistivity to pore fluid resistivity) is not a standard measurement although it provides a critical basis for calculating fluxes of electron donors and acceptors (e.g., D'Hondt et al., 2004). Because the formation factor and complete suites of metabolic reactants and products are not routinely determined, those biologically relevant properties that *are* routinely measured (e.g., the concentration of sulfate) are not consistently usable for quantitative studies. In situ temperature is not a standard measurement although it is necessary for all quantitative assessments of microbial activities, for determination of proper culturing conditions, and for interpretation of all microbiological results.

At present, the intervals at which interstitial water is sampled are often so coarse that rates of biogeochemical processes cannot be accurately quantified. A ten-meter interval (once per core) is appropriate for defining concentration profiles over hundreds of meters of depth but does not provide enough resolution to accurately quantify rates in intervals of special interest. For example, to quantify the respiration for an entire subseafloor sediment column, interstitial water samples must be taken at the meter scale or less for the first several meters below seafloor and for the last several meters above the interface with the basement.

Microbial cell counts were identified as a standard measurement by the IODP SAS in 2007. Counts of appropriately fixed samples will allow significant progress in mapping geographic distributions of subseafloor sedimentary biomass and in constraining global estimates of subsurface biomass. In practice, routine cell counts are unlikely to be useful for most non-sedimentary (e.g., basalt and gabbro) samples, which are commonly extensively contaminated during drilling. For all subseafloor environments, ensuring that cell counts are reproducible and comparable from one expedition to another will require clearly defined sample handling procedures and parallel sampling for post-expedition verification of results.

Essential modifications of the standard measurements include routine measurements of the formation factor and of the concentration of DIC. This will allow quantification of (1) in situ pH (from [ALK], [DIC], in situ temperature and in situ pressure) and (2) gross heterotrophic (organic-fueled) respiration in the subseafloor sediment column of most drill sites (using the approach outlined by Wang et al., 2006).

Contamination testing was identified as a standard measurement by the IODP SAS in 2007. To ensure the quality of microbiological legacy samples and routine cell counts, perfluorocarbon tracer (PFT) must be routinely injected into the drilling fluid for all holes from which microbiological samples are taken. The concentration of the tracer must then be measured for the same stratigraphic horizons from which microbiological samples are taken. Recommendations for implementation of contamination testing will be discussed later in this report.

The highest priority new standard measurements are

- Routine measurement of in situ temperature (e.g., ADARA, DVTP)
- Routine measurements of formation factor in sediment
- Measurement of DIC concentration as a standard interstitial water measurement (in combination with measurement of formation factor, [ALK], [Ca^{2+}] and [Mg^{2+}]).

Strategies for legacy samples

Appropriately selected legacy samples will allow significant progress on mapping subseafloor biomass, diversity, community composition, habitability and activity. Here, we describe the scientific value of such samples. Appropriate sampling techniques are described in a later subsection.

Bulk sediment samples frozen to -80C are necessary for post-expedition analyses of biomass, diversity and community composition (via nucleic acid analyses and organic biomarker analyses). They are also useful for some analyses of potential *in situ* microbial activities, e.g. hydrogenase activity (Soffientino et al., 2006) or other enzymatic assays. Analyses of nucleic acids provide the ultimate basis for determining the diversity and phylogenetic composition of the total community via DNA (Inagaki et al., 2006) and of the active community via RNA (Sørensen and Teske, 2005). Organic biomarkers (e.g., phospholipids) provide independent proxies for biomass (through their abundance) and for community composition (through their structures) (Biddle et al., 2006). Furthermore, since these investigative techniques are developing rapidly, storage of legacy samples at

-80° C will insure that a useful archive will be available from *all* pertinent IODP expeditions.

Sediment samples fixed with formalin and frozen to -80C are necessary for post-expedition determinations of total cell abundance (e.g., SYBR Green I counts) and active cell abundance and composition [e.g., CARD-FISH counts of bacteria and archaea (Schippers et al., 2005)].

To provide sufficient material for multiple technical approaches and for analyses by multiple laboratories, multiple sub-samples should be taken for each category (frozen bulk sediment or frozen formalin-fixed sediment). Such sub-samples are necessary to guarantee reproducibility of results and to allow complementary analyses.

Greatly increased understanding of the redox (energetic) habitability of both basalts and sediments can be achieved by routinely taking samples appropriate for post-expedition quantification of the abundance and redox states of sulfur, iron and carbon in solid phases. Relevant analyses include measurements of ferrous/ferric ratios and combustion oxygen demand (Perks and Keeling, 1998). Aside from redox habitability, the potential for advances with routine basement samples or measurements is greatly limited by the near-impossibility of drilling hard rocks without contamination of the microbial communities and formation fluid. Therefore, outside of these redox habitability measurements, the legacy sampling that we recommend is, at present, limited to sedimentary environments.

Routine sampling of interstitial waters from sediment would allow post-expedition measurements of several dissolved microbial reactants and products, including short-chain fatty acids (acetate, formate, lactate), dissolved inorganic carbon, sulfide, ammonium, iron, manganese and nitrate. When combined with standard measurements (e.g., dissolved sulfate, alkalinity, calcium, magnesium, etc.), these samples will allow (1) thermodynamic studies of microbial energetics in the deep subseafloor ecosystems (e.g., Hoehler et al., 2001; Wang et al., 2006) and (2) quantification of subseafloor reaction rates (if combined with shipboard measurements of the formation factor). However, the task force recognizes the complexity of appropriate sampling and storing interstitial water samples for these various compounds. Consequently, we suggest that inclusion into detailed sampling requests or APLs by the subsurface life community may be a better approach to meeting this level of detail for interstitial water samples.

We have not included +4°C (refrigerated) or room temperature samples as part of the suggested legacy program because artifacts due to sample oxidation and pressure changes significantly hamper the long-time viability and variability of such samples. As with expanded interstitial water sampling, we suggest that the best approach for taking refrigerated or room-temperature samples may be incorporation into detailed sampling requests or APLs by the subsurface life community.

The highest priority legacy samples for studies of subseafloor life are:

- -80C bulk sediment for molecular studies of diversity, community composition and biomass
- formalin-fixed samples for post-cruise censuses of total cells, active cells and community composition
- solid-phase samples of sediment and basement for studies of energetic habitability

Strategies for targeted addition of subsurface life studies to IODP expeditions

With carefully targeted studies, understanding of subseafloor life can be advanced by addition of biosphere-focused subprojects attached to IODP expeditions with non-biosphere primary objectives. Depending on the study, such advances will require modest IODP investment by the addition of sites, holes and/or biosphere-focused shipboard scientists. To ensure that these "piggy-back" projects are defined and protected fully

enough to maximize their success, such studies would probably best be advanced *via* submission and acceptance of Ancillary Program Letters.

Addition of one or more projects to individual expeditions will allow shipboard scientists to understand how environmental properties (e.g., lithology, temperature) specific to the targeted environment control subseafloor microbial diversity, community composition, and activities and effects on the environment (e.g., sediment diagenesis and fluxes of chemicals between the sediment and the ocean). Successful addition of such projects to multiple expeditions will advance general understanding of the distribution of subseafloor life (activities, diversity and biomass) and may advance understanding of the environmental limits to life on Earth.

The potential nature of such advances will vary from one expedition to another, depending on the environment to be drilled. Consequently, discussion of specific advances requires reference to specific expeditions or environments.

For example, understanding of environmental controls on subseafloor diversity, biomass and activity can be advanced by incorporation of "piggy-back" projects into expeditions presently proposed for the Bering Sea, the equatorial Pacific, the Nankai area, the Marianas Forearc and Costa Rica mud mounds.

1) Bering Sea drilling will provide a unique opportunity to test the control of oceanographic properties on global distributions of biomass and heterotrophic activity in subseafloor sediments. The Bering Sea sites presently scheduled for drilling will sample the sediments beneath extremely high-productivity waters. No oceanographically equivalent sites are scheduled for drilling elsewhere. Such a project might be best advanced by inclusion of one or two appropriate scientists on the expedition (to respectively take microbial samples (for biomass and nucleic acids) and take interstitial water samples. The sampling strategy would require high-resolution (decimeter-scale to meter-scale) samples near the seafloor and the basement interface and regular sampling at 10-m scales throughout the sediment column of representative sites. Given the need for relatively continuous records to meet the paleoceanographic objectives of the expedition, the high-resolution near-seafloor sampling might be best provided by taking a shallow (20 to 30-m) biosphere-dedicated core at representative sites.

2) Equatorial Pacific drilling will provide an excellent opportunity to test how Milankovitch-scale (glacial/interglacial) paleoceanographic changes and resultant variations in lithology shape the compositions of present-day subseafloor communities. This might best be done with a sampling strategy similar to that outlined for the Bering Sea drilling (above).

3) Effects of tectonic activity on subseafloor habitats and ecosystems could be modestly advanced by incorporation of biological studies into drilling efforts in the Nankai area.

4) Explicit incorporation of biological studies into Marianas Forearc drilling would advance understanding of how subseafloor microbial ecology is coupled to plate-tectonic cycling via the effects of subduction-zone fluid flow on subseafloor microbial communities and activity. It would also provide an opportunity to search for a pH limit to subseafloor life.

5) Drilling of the Costa Rica mud mounds will provide an opportunity to examine the effect of temperature on organic degradation and its consequences for the subseafloor microbial ecosystem.

In each case, non-routine samples, appropriate shipboard scientists (microbiologists, biogeochemists, physical property specialists) and perhaps dedicated holes or sites will be required

Strategies for dedicated expeditions

A major advance in understanding of subseafloor life within the next five years will require at least one dedicated IODP expedition per year. Improvement of routine

measurements and samples and "piggy-back" projects are important but cannot substitute for dedicated expeditions.

First and foremost, only dedicated expeditions can allow a sustained attack involving a large number of specialists, multiple dedicated sites, and many days of operation. Many objectives in study of subseafloor life require focused attention by scientists from diverse fields. These include hydrologists, physical-property specialists, sedimentologists or igneous petrologists, and biostratigraphers in addition to microbiologists and biogeochemists. Second, dedicated expeditions are necessary to meet any objectives that require operation in environments not targeted by other IODP proposals.

Dedicated expeditions are required to determine the energetic limits to life in subseafloor sediments and the distribution of organisms and activities at energetic extremes. Examples of such extreme environments include sediments in mid-ocean gyres, where concentrations of organic matter and electron donors are extremely low, and high-latitude upwelling regions, where abundances of organic matter and electron donors are extremely high but coupled at great sediment depths to extremely low concentrations of electron acceptors.

Dedicated expeditions are necessary to understand fully the influence of fluid flow (e.g., hydrothermal transport) and of hydrocarbons (petroleum, gas) on sedimentary communities and activities. For example, the energetics of microbial communities in hydrocarbon deposits and the roles of microbial processes in the generation and destruction of hydrocarbons are poorly known. Concentrations of electron acceptors and thermodynamic limits to microbial activities may be especially significant. Drilling in these environments may require the riser capabilities of the *Chikyu*. These objectives may provide a significant opportunity for IODP-industry cooperation.

Dedicated expeditions are necessary to fully understand lithologic control of subseafloor communities and activities. Most fundamentally, if the same horizon is sampled repeatedly in different holes and at multiple sites, is the diversity and composition of the community always the same? At a more exotic level, a dedicated expedition could identify the extent to which "hydrothermal" sediment just above basement-sediment interfaces sustains and is modified by microbial redox cycling of metal and sulfur in concert with introduction of O_2 and NO_3^- from seawater in the upper basement.

Dedicated expeditions are necessary to understand the evolution of habitability in basement water-rock reaction zones as a function of crustal age and in response to ventilation by subseafloor circulation. Understanding of the interplay between age, ventilation, and the basalt-hosted biosphere will require drilling in on-axis, open-flow environments; off-axis, closed-flow (sediment-sealed) environments; and off-axis, open-flow environments. Seamounts are natural bioreactors and a major focal point for these studies; both hot systems and old, cold systems are hydrologically active and thus foci for microbial activity. Dedicated expeditions are required to study their role in the evolution of redox habitability, ventilation of the subseafloor ocean, and effects on the distribution of subseafloor life in both basaltic crust and sediments.

Studies of microbial life in ridge crest environments (both high-temperature and low-temperature serpentization zones) will also be aided by dedicated expeditions, as will studies of subseafloor life in arc, back arc and plume-related marine volcanic systems, including volcanogenic sediments.

Some of these problems will require multiple expeditions. Detailed mapping of microbial ecosystems at an active ridge complex provides one example. Determination of the energetic limits to organic-fueled (heterotrophic) subseafloor life will require at least two expeditions, one focused on a region with extremely low organic abundances and one to sites with extremely high abundances of electron donors but a general absence of electron acceptors such as O_2 and SO_4^{2-}. Because the scientific gains from well-planned individual expeditions are likely to be great, expeditions that collectively address these kinds of broader problems need not be rigidly linked.

Other problems will require CORK-based microbial observatories, particularly in basement environments where mineral habitability is the only microbially relevant property unlikely to be altered or contaminated at the time of drilling.

Implementation

Implementation of standard measurements

New standard measurements.—The highest priority new standard measurements are

- Routine measurement of in situ temperature (e.g., ADARA, DVTP)
- Routine measurements of formation factor in sediment
- Measurement of DIC concentration as astandard interstitial water measurement (in combination with measurement of [ALK], [Ca^{2+}], [Mg^{2+}] and formation factor

IODP protocols already exist for in situ temperature measurements. At least four to five in situ temperature measurements should be made at different depths in the sediment at each site.

Formation factor should be measured at the same frequency or higher than the IW sampling interval. Formation factor should be measured as described in the ODP Leg 201 Initial Report (Shipboard Scientific Party, 2003).

To quantify total heterotrophic respiration at each site, concentrations of dissolved inorganic carbon [DIC], Ca^{2+} and Mg^{2+} should be measured at relatively high resolution (one- to two-meter intervals) for the first 20 to 30 meters below the seafloor (mbsf) and the last 20 to 30 meters above the sediment/basalt interface. A sample should be taken at about one mbsf (ideally, two or more samples would be taken in the first 1.5 mbsf). For sediment depths greater than 20 to 30 mbsf and farther from basement than 20 to 30 meters, it should be measured at the same 10-meter interval as standard IW samples. DIC concentrations can be measured with the existing carbonate coulometer in the shipboard geochemistry laboratory using the Leg 201 protocol (Shipboard Scientific Party, 2003). Concentrations of Ca^{2+} and Mg^{2+} can be measured with the shipboard ICP (Shipboard Scientific Party, 2003).

Implementation of standard contamination tracer measurements.—Standard Perfluorocarbon tracer (PFT) measurements should be undertaken on holes and stratigraphic horizons where microbiology legacy samples are taken (sediments). The PFT should be introduced and measured as described in the Leg 201 Initial Report (House et al., 2003).

Implementation of standard cell counts.—The IODP SAS declared cell counts a standard measurement in 2007. Such counts have historically been done on ODP and IODP samples with Acridine Orange (e.g., Parkes et al., 2000). However, Acridine Orange is no longer the best choice for nucleic acid staining of subsurface samples due to the development of more specific fluorochromes that produce a much brighter signal with much lower background fluorescence. We strongly recommend that all standard cell counts be done with SYBR Green I, not Acridine Orange. Samples stained with SYBR Green I are characterized by low background fluorescence and relatively slow signal quenching. Standard sedimentary cell counts should be done at intervals similar to IW samples (one to two-meter intervals for the first 20 meters, 10-m intervals at greater depths, with a sample taken at one mbsf.

Standard cell counts will be useless unless the results are reproducible by independent observers. The simplest way to minimize variation in observer bias would be to have all standard cell counts done by the same observer and to have representative subsamples

checked by independent observers. We strongly recommend that parallel sub-samples be taken as legacy samples for post-expedition verification of results by independent laboratories.

Recommended technological developments for standard measurements.—IODP or its funding organizations should fund development and testing of one or more dissolved contamination tracers that is less volatile in the environment than the currently used PFT, but easily volatilized for analysis. The present PFT measurements have relatively high blanks because the tracer is so easily volatilized during handling of microbiological samples. For most environments (excluding very high pH environments), a dissolved contamination tracer volatilized by addition of a strong base may be ideal. An acid-liberated tracer is not recommended for high-carbonate sediment samples, due to the large amount of CO_2 that would be simultaneously liberated. A temperature-liberated tracer would not be ideal because it could not be used for high-temperature subseafloor environments.

IODP or its funding organizations should fund development and testing of membrane-inlet mass spectrometry (MIMS) or similarly related gas-chromatographic technology for quantification of in situ dissolved concentrations of methane and other gases. Use of MIMS technology, with void-space CH_4 concentrations normalized to O_2 and Ar concentrations, using the approach of Spivack et al. (2006) will allow routine shipboard quantification of in situ dissolved gas concentrations without the need for complex downhole technologies (such as pressurized core recovery).

Implementation of legacy samples

The highest priority legacy samples for studies of subseafloor life are:

- -80 bulk sediment for molecular (DNA, RNA, biomarker) studies of diversity, community composition and biomass
- formalin-fixed samples for post-cruise censuses of total cells, active cells and community composition
- solid-phase samples of sediment and basement for studies of energetic habitability

For the -80C bulk sediment samples, we recommend that multiple paired subsamples, e.g., four sterilized large-volume (60cc?) cut-off syringes, be taken from the same central portion of the same cut surface. To take these samples, the core should be cut perpendicular to the core liner. The outer edge of the core and any fractures or disturbed core should be avoided entirely by these samples. These samples can be taken in association with IW samples (from a cut core surface that faces the IW sample). These samples should be taken as quickly as possible after core recovery. The cores should be refrigerated until they are sampled. Where cut-off syringes cannot be inserted because the sediment is too hard, we recommend that four adjacent 5-cm-thick whole rounds of the core be cut. If subsequent demand indicates that this volume of legacy material is inadequate, then volume should be increased, e.g., to eight syringes or eight five-inch whole rounds. These legacy samples must be taken routinely, even on legs where shipboard scientists take other samples for molecular studies.

For the legacy samples to be fixed in formalin and frozen (-80C) for microscopic census of total cells (e.g., SYBR Green I, AODC), active cells (e.g., FISH assays) and other whole-cell analyses, we also recommend that multiple subsamples be taken. Similar to the bulk sediment samples, we recommend that four such samples be taken with sterilized cut-off 3cc syringes. Individual 1cc aliquots of sediment should be transferred into an equal volume of formalin (2-3%) diluted in a sterile buffer of comparable in situ salinity (PBS is standard or 2.5% NaCl). Fixed sediment for in situ hybridization (FISH) can be stored 6 hours-overnight at 4ºC and then transferred to -80ºC for long(er) term

storage (or flash frozen in liquid nitrogen). These samples can also be taken in association with IW samples (from a cut core surface that faces the IW sample). These samples should be taken from the same whole round as the -80C bulk sediment samples or an immediately adjacent whole round. As with the -80C bulk sediment samples, the outer edge of the core and any fractures or disturbed core should be avoided entirely by these samples. These samples should be taken as quickly as possible after core recovery. The cores should be refrigerated until they are sampled. These legacy samples must be taken routinely, even on legs where shipboard scientists take other samples for biomass studies.

The principal advantages of this formalin-based approach to legacy samples are increased longevity of samples for certain categories of microscopic study (such as FISH analyses) and the minimal shipboard sample-handling requirement of the approach. The latter advantage is a key feature if the samples are to be collected by non-dedicated personnel. However, it should be recognized that a systematic study of cell loss using this method has not been done; the accuracy of cell counts on samples fixed and frozen in this manner should be tested before the approach is used routinely on IODP missions.

For post-expedition studies of energetic habitability, a piece of core should be taken every 10 m, bagged in an N_2-flushed atmosphere or, if chips, sealed in a 12-mm evacuated tube and frozen at -80C. These samples are necessary for post-expedition studies of easily altered biogeochemical properties, such as sulfur speciation, ferrous/ferric ratio, concentrations of mineral-bound CO_2, H_20 and sulfur, and combustion oxygen demand (total oxidizable content of sediment). For sediment, these samples can be taken from squeeze cakes (the sediment that remains after squeezing sediment for interstitial water).

All frozen samples must be shipped from the drilling platform to their eventual destination (IODP repository or individual scientist) with temperature loggers to verify the temperature history of the shipping.

Recommended technological developments for legacy samples.—Two technological developments will significantly advance the potential for scientific yields from microbiological legacy samples. IODP or its funding organizations should fund development and testing of both developments.

First, the long-term consequences of formalin treatment and storage at -80C for microscopic assays of total biomass (e.g., SYBR Green I), active biomass (e.g., FISH) and other whole-cell analyses (e.g., secondary ion mass spectrometry) must be quantified by multi-year studies.

Second, a wide array of bulk-sediment sample processing techniques for genomic analyses must be rigorously tested and compared, in order to provide baseline techniques for analysis of subseafloor materials. This exercise could be done by a competition with a request for proposals focused on extraction techniques; the competition should require that the extractions by different techniques be done on parallel samples and that the results be provided for calibration exercises.

A less crucial technological issue is that sampling and medium to long-term storage of non-frozen, non-fixed samples continues to pose problems. A commonly accepted method to method is to store samples anerobically under N_2 gas in heat-sealed aluminum "H_2S-bags" or gas impermeable trilaminate bags (Cragg et al. 1992). Without the inclusion of an extra chemical oxygen scrubber, oxidation very often occurs time periods of a year (Lin, Hinrichs, & Biddle, unpublished data). The addition of the commercial chemical oxygen scrubbers, however, can add substantial amounts of hydrogen. Further exploration of proper sample packing and long-term storage will be needed before non-frozen non-fixed samples can be considered for legacy sampling.

Recommended requirements for microbiological studies of IODP materials (shipboard and post-expedition)

The scientific value of molecular results and cultured microbial strains ultimately depends on their accessibility to the international scientific community. Public DNA sequence repositories are particularly invaluable resources for characterizing the diversity and global distribution of subseafloor life. To fully maximize the scientific benefits from this data, we recommend that the IODP standardize the reporting of key metadata during the submission of sequence/ metagenome data to public databases. This contextual data should at a minimum include information regarding IODP expedition number and site, sample location- lat/lon, depth, sample processing (PCR primers used, PCR cycle number, extraction type RNA/DNA), and the relevant publication (IODP cruise logs as well as peer reviewed literature).

Highest priority recommendation

- IODP should require routine submission of all sequence data and standardized contextual data to an appropriate international database, such as *GenBank,* the *European Molecular Laboratory Nucleotide Sequence Database,* or the *DNA Databank of Japan*.
- IODP should require all published culture strains to be deposited in publicly accessible culture collections for ready access by the international scientific community.

Second priority recommendation

- *JCORE* and *JANUS* should be modified for post-expedition inclusion of designated data

Steps for encouraging subsurface life studies of IODP materials

Subsurface life studies can only be undertaken on IODP expeditions if potentially interested scientists know of the opportunity far enough in advance to successfully apply for participation.

Several mechanisms are appropriate for inclusion of subsurface life studies on IODP expeditions. These mechanisms include sample requests, shipboard scientist applications Ancillary Program Letters (APLs), and IODP proposals dedicated to study of subseafloor life. Of these mechanisms, APLs are a particularly crucial tool for adding subsurface life studies to IODP expeditions scheduled with any primary objective. They force proponents of add-on projects to refine and justify their projects. They provide a clear mechanism for adding IODP resources (e.g., additional drilling time and transit time, dedication of shipboard science berths, etc.) to expeditions scheduled with different primary objectives. They protect the resources provided for the add-on projects from being sacrificed to other objectives during the course of the expedition.

None of these mechanisms are known to any significant fraction of the environmental microbiology and biogeochemistry communities. IODP must take concrete steps to make their existence more transparent to those communities. We recommend that the following minimum steps be taken by IODP MI:

- Carefully constructed advertisements should be placed in appropriate journals and at appropriate meetings. These advertisements should clearly state the likely expedition schedules well in advance. They should succinctly state the three principal scientific themes of the IODP Initial Science Plan (ISP). They should identify three categories of potential participation (sample requests, shipboard scientist applications, Ancillary Program Letters). They should include generic timelines for APL submissions, shipboard scientist applications and sample requests, and a link to likely expedition schedules far enough in advance that APLs can be developed and submitted. They can also advertise routine samples

and routine measurements. In principal, all three principal themes of the IODP ISP could be advanced in this manner.

- Microbiology must be included as a research category and frozen samples must be included as an archived sample category in IODP sample request forms.
- Information about microbiology and archived frozen samples (e.g., from ODP Legs 201, 204 and any other expeditions where such samples were/will be taken) must be provided on easily accessible IODP webpages that are clearly linked to the sample request forms.
- Post-314 sample request forms must be made easily accessible [when we checked the page at our Sept Task Force meeting, the text of the introductory page was in German and the page did not allow us to log in (after registration, no log-in link appeared)— http://smcs.iodp.org:8080/smcs/. When checked by a Task Force member more recently (Feb 12, 2008), the site first loaded in Japanese, but switched to English when a random button was clicked.]
- The new visitor page of the IODP web site needs a vision statement that concisely states the three major themes of the IODP Initial Science Plan, including study of the deep biosphere and the subseafloor ocean.
- APLs must be clearly identified as a proposal option on the IODP website and a clear pathway must be provided that links the new visitor page to the APL option. As with the advertisements, the APL-related webpages should include generic timelines for APL submissions, shipboard scientist applications and sample requests, and a link to likely expedition schedules far enough in advance that APLs can be developed and submitted.

These steps will provide an information exchange and alert system intended to lead to APLs for one or two subsurface life projects per year, plus routine subsurface-life shipboard scientist applications and routine subsurface-life sample requests.

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