Integrated Ocean Drilling Program
Microbiology Working Group

Members

Heribert Cypionka, Institut für Chemie und Biologie Des Meeres
Katrina Edwards, Woods Hole Oceanographic Institution
Fumio Inagaki, JAMSTEC
Kenji Kato, Shizuoka University
Craig Moyer, Western Washington University
Kenji Nanba, University of Tokyo
David C. Smith, University of Rhode Island (co-chair)
Ken Takai, JAMSTEC (co-chair)
Peter Wellsbury, University of Bristol

Our Charge:

iPC Consensus 3-17: The iPC requests that iSciMP form a microbiology working group to examine issues related to the conditions and duration of sample storage, to make recommendations about the importance of patent rights, to formulate requirements for data reporting and publications, and to identify ways to attract more microbiologists to the program.

Note from Microbiology Working Group co-chairs: Prior to assembling the Microbiology Working Group, a Memorandum of Cooperation between the U.S. (NSF) and Japan (MEXT) was signed. The memorandum addresses issues concerning intellectual property and data rights and therefore discussions of these topics were not considered by this working group. The relevant sections of the memorandum signed on 22 April 2003 are below.

> **Section VII. Data, Information, Intellectual Property Rights**
> The Agencies take necessary measures to assure that all data, samples, and scientific and technical results of the Program's scientific and engineering activities are made widely available to the international scientific community and to the public through customary channels and in accordance with the normal procedures of the Agencies, or an identified by the SAS. Such measures should be taken in accordance with the respective laws and regulations of Japan and the United States.
>
> Information transmitted by one Agency to the other under this Memorandum is expected to be accurate to the best knowledge and belief of the transmitting Agency which may not be liable for the content or issue of such information.
>
> Protection of intellectual property and rights thereto resulting from

scientific research activities conducted under the auspices of this Memorandum will be addressed as set forth in Annex IV to the Agreement between the Government of Japan and the Government of the United States of America on Cooperation in Research and Development in Science and Technology, signed at Toronto on June 20, 1988, and extended by the Protocols done at Washington on June 16, 1993, on June 16, 1998, on March 19, 1999, and on May 19, 1999, and extended and amended by the Protocol done at Washington on July 16, 1999.

ANNEX IV Annual Member Contributions and Rights (final two paragraphs)

An IODP member with at least one participation unit may maintain the same rights in data as the Agencies for activities conducted using the IODP science operations funds.

An IODP member with at least one participation unit is to have the right to a royalty free license for all patents resulting from developments supported by the IODP science operations funds.

1) Introduction

Interest in microbes inhabiting the marine deep subsurface has increased dramatically towards the end of the Ocean Drilling Program. As a result of this interest, microbiology became better integrated into the program. This culminated in the establishment of a well equipped microbiology laboratory onboard the JOIDES Resolution and the participation of more and more microbiologists. The purpose of this document is to lay out how IODP can capitalize on the knowledge gained during ODP and further integrate microbiology into the new program.

In response to iPC Consensus Statement 3-17, a Working Group of microbiologists was formed. This group is co-chaired by the two microbiologists that serve on iSciMP (Smith and Takai). The other members are expert in various aspects of environmental microbiology and have previous experience with the Ocean Drilling Program. The working group did not meet in person but rather worked on this document via email. Many of the issues described in the request from iPC have evolved independently, and this WG Report helps consolidate and formalize these practices, as well as make new recommendations to help ensure that the scientific goals articulated in the Initial Science Plan of the IODP ("Earth, Oceans, and Life") are able to be realized.

While the WG appreciates the significant progress the ODP has made in microbiological studies, they also feel that it is the IODP's responsibility to ensure that the microbiological measurements are continually made, and not on an ad hoc basis. Tremendous amounts of knowledge have been gained in other shipboard laboratories

(e.g., the interstitial water program) even on legs for which those measurements are not fully associated with the leg objectives. It will only be after 5-10 years of continual and routine microbiological sampling and analysis that benefits will begin to become apparent. The implementation of the following recommendations will help us to reach this goal.

2) Sample Collection

A wide variety of analyses in support of the study of microbes in the deep subsurface have been employed on subsamples of recovered cores. Specific handling procedures are required for the various downstream procedures. In all cases, avoiding contamination of the cores with non-indigenous microbes, either during the drilling process or the subsequent subsampling is of paramount importance. Subsamples used for DNA and biomarker analyses should be frozen (preferably in liquid nitrogen, -196°C) as soon as possible after their isolation from the core. Subsamples that are used for subculturing must be protected from dramatic increases in temperature or from exposure to oxygen.

Subsampling Strategies:

. Subcore with sterile syringe. Ideally, a subcore is taken directly from the end of a core section on the catwalk. To reduce the potential for introducing contamination, the core is broken after the core liner is cut. If the core is cut with a blade or wire, the exposed end of the core must be scraped with a sterile blade prior to inserting the syringe. The ends of syringes (1, 3, 5, 10, or 50 mL) are cut off and used to take mini-cores from the uncontaminated interior of the cores. For indurated sediments, the syringes are pounded in to the center of the core using an adaptor developed at Bristol University. This method has been used extensively for the direct cell count samples. It is also very useful for samples for subculturing or molecular biology. This method yields an uncontaminated subcore that can be assayed directly or stored for later analysis.

. Whole round cores. Whole round samples (typically 5 or 10 cm in length) are cut on the catwalk, in the lab or in a cold room. The core liner is cut using the standard cutter and the core itself is broken or cut using a spatula or a wire. The whole rounds require additional work to remove the outer edge which is contaminated by drilling fluid.

. Hard rock samples. Individual rock pieces are sampled by paring away the contaminated outer edge using sterilized (flame or autoclave) chisels. The clean interior can be further processed by crushing using a stainless steel percussion mortar.

2) Sample Storage

Requirements for sample storage conditions are dependent upon the downstream assay. The following considerations are pertinent to samples that will be used in a more

immediate manner (i.e. shipboard sample request) as well as those that will be shipped to shore-based laboratories or repositories for future analyses. It must be noted that even samples that are stored properly are not useful indefinitely and these samples are not a long term archive.

a. Frozen samples. Frozen samples are used for nucleic acids, lipid biomarkers, amino acids etc. These samples should be collected as soon as possible and immediately frozen, ideally in liquid nitrogen. This works best with subsamples taken in syringes as the core liners crack during freezing and increase the potential for contamination. The samples can be stored in liquid nitrogen or transferred to ultra low freezers (- 80°C). It is critical that the samples remain frozen until analysis. This includes shipping on dry ice (- 78°C). It is essential that the materials not thaw during transport, even briefly. Samples stored in ultra-low freezers can be maintained in an anaerobic environment by adapting the method of Cragg, *et al*., 1992).

b. Anaerobic samples. Samples that will be used for subculturing should be stored in an anaerobic environment until used. This can be achieved using oxygen scrubbers and gas impermeable trilaminate bags (Cragg, *et al*., 1992).

c. Chemically fixed samples. Samples used for microscopy (e.g. direct cell counts, fluorescent in situ hybridization, microautoradiography) are chemically stabilized in aldehyde solutions (formaldehyde, glutaraldehyde) and stored at 4 °C. Again, the particular downstream assay dictates the particular details necessary in the fixation process.

Because maintaining the proper temperature for the particular downstream analysis is essential, a temperature logger included in the shipping container can provide the researcher with the thermal history of the samples during transit.

The above discussion leads to the following Recommendation addressing the routine collection and storage of samples for microbiological analyses.

Recommendation 1: IODP should establish a repository for samples routinely collected and stored appropriately for subsequent microbiological analysis. The samples should be taken in sterile syringes (50 cm^3 capacity) as soon as the core arrives and stored as described below depending on the subsequent analysis.

a. Samples for nucleic acid analysis should be placed immediately in liquid nitrogen and transferred to ultra-low freezer or liquid nitrogen on board for storage. Alternatively, whole round samples used for this purpose should be placed directly in an ultra-low as soon a possible.

b. Samples taken for culturing work should be transferred to gas-tight trilaminate bags containing an oxygen scrubber, heat-sealed and stored at 4 °C.

c. Samples for microscopy should be preserved with an aldehyde solution (electron microscopy grade glutaraldehyde or paraformaldehyde) and stored at 4 °C.

3) Drilling Methods

Some analyses are most likely compromised by the depressurization upon ascent. To date, all microbiological samples have undergone depressurization prior to subsampling. Therefore, by default, all microorganisms that have been cultured from recovered cores can withstand exposure to a pressure of 1 atmosphere. The currently unavoidable depressurization precludes us from culturing microorganisms that are sensitive to the reduced pressure. The continued development of pressure retaining core barrels, with the ability to subsample at the in situ pressure (e.g. HYACE/HYACINTH) is extremely valuable for microbiological studies and should be supported.

Even more critical than changes in pressure are increases in temperature. This can be minimized by expediting the removal of the core from the core barrel and giving high priority to subsampling for microbiological samples. Core processing on board should be optimized to recover the core as quickly as possible in order to minimize increases in temperature. IODP should also explore the methods for insulating the core after removal from the core barrel. Because all temperature considerations are relative to the in situ temperature, better measurements of the downhole temperatures are essential.

Quality control issues have been addressed by introducing methods for quantifying the intrusion of drilling fluid (Smith, *et al*., 2000a). The judicious use of these methods are essential to maintaining scientific integrity of our observations. Overuse of the perfluorocarbon tracer results in yielding excessively high background levels in the laboratories which results in lowering the sensitivity of the method. As with interstitial waters samples, experience has shown that the use of the extended core barrel (XCB) produces cores of inferior quality (Smith, *et al*., 2000b) for microbiological study. Extending the range of the more desirable hydraulic piston core (APC) by "drilling over" should be used whenever possible. While this comes at the expense of time and equipment, it yields samples that are of sufficiently high quality for microbiological analyses. Hard rock samples collected with the rotary core barrel (RCB) are more problematic with respect to contamination issues. In practice, the fluorescent microspheres appear to be a more appropriate tracer for hard rock samples. The single test using the diamond core barrel system (DCB) yielded a clean sample. To date, the motor driven core barrel (MDCB) has not been tested. In general, for all drilling tools, larger diameter cores will yield more uncontaminated material for a given length of core and is more desirable. This will also yield more material from a specific horizon and allow for more the analysis of samples at higher vertical resolution.

Recommendation 2: Drilling methods that yield cores of optimal quality for microbiological studies should become standard.

a. Optimization of core processing with the goal of minimizing increases in temperature and exposure to oxygen should be implemented.

b. Continued performance, and further improvements to the methods for contamination testing (House*, et al*., 2003) while coring.

c. Routine use of the drill over method extends the useful range of the APC method and provides superior results for microbiological studies and should be implemented.

d. The continued development of the pressure retaining core barrel, and subsequent handing under in situ pressures is highly valuable to the microbiology research and must be given highest priority.

4) Data Reporting and Publications

Microbiologists are required to follow the IODP Sample and Data Policy as any other group. Because microbiologists generate some types of samples and data that are unique to their field, however, some additional issues need to be addressed.

a. Sequence data. The sequencing of nucleic acids has become the standard method for identifying microorganisms. The usefulness of the data resides in the ability to compare sequences. This is accomplished by submission of sequences to internationally recognized, publicly accessible, databases (below). In general, microbiological journals require submission of sequence data to one of these databases prior to publication. These requirements are specifically stated in the 'advice to authors'. These statements from FEMS Microbiology Ecology[1] and Applied and Environmental Microbiology[2], two pertinent journals, are included in the footnotes.

DDBJ
Center for Information Biology and DNA Data Bank of Japan
National Institute of Genetics
111 Yata, Mishima, Shizuoka 411-8540, Japan;
telephone, 81-559-81-6853
fax, 81-559-81-6849
e-mail, ddbj@ddbj.nig.ac.jp
URL, http://www.ddbj.nig.ac.jp

EMBL
EMBL Nucleotide Sequence Submissions, European Bioinformatics Institute
Wellcome Trust Genome Campus
Hinxton, Cambridge CB10 1SD, United Kingdom
telephone, 44-1223-494499
fax, 44-1223-494472

e-mail, datasubs@ebi.ac.uk
URL, http://www.ebi.ac.uk.

GenBank
National Center for Biotechnology Information
National Library of Medicine, Bldg. 38A, Rm. 8N- 803
Bethesda, MD 20894
telephone, 301-496-2475
fax 301-480-9241
e-mail, info@ncbi.nlm.nih.gov
URL, http://www.ncbi.nlm.nih.gov.

b) Culture isolates. A common goal for many microbiologists is to obtain pure cultures of microorganisms in order to perform detailed studies on their physiological capabilities, produce specific enzymes or metabolic byproducts etc. It is common practice to place subsamples of the cultures into publicly accessible culture collections. The leading journals in the field advocate this practice[2]. In keeping with the open, international cooperation established during the previous decades of scientific ocean drilling, IODP should require that cultures of microorganisms isolated from cores be deposited in a publicly accessible culture collection (e.g. Takai, *et al*., 2003).

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
(703) 365-2700
E-mail news@atcc.org
http://www.atcc.org

Japan Collection of Microorganisms
RIKEN (The Institute of Physical and Chemical Research)
2-1 Hirosawa, Wako, Saitama 351-0198, Japan
Phone: +81 48 467 9560
Fax: +81 48 462 4617
E-mail: curator@jcm.riken.go.jp
http://www.jcm.riken.go.jp/

German Collection of Microorganisms and Cell Cultures (DSMZ)
Mascheroder Weg 1b
38124 Braunschweig
GERMANY
Phone:+49 (0) 531-2616-0
Fax:+49 (0) 531-2616-418
http://www.dsmz.de

Recommendation 3: IODP should adopt policies to those that are already firmly established within the international community of microbiologists for the exchange of culture and sequence data.

a. Unique nucleic acid sequence data derived from cores and published in IODP publications or scientific journals must be submitted to an internationally recognized, publicly accessible database (e.g. DDBJ, EMBL and GenBank).

b. Subcultures of organisms derived from cores and published in IODP publications or scientific journals must be deposited in at least two internationally recognized, publicly accessible culture collections (e.g. ATCC, JCM and DSMZ).

5. Increasing Participation

Microbiologists increased their participation towards the end of ODP. Further increasing the participation of microbiologists in IODP will lead to a more rapid understanding of the role of microorganisms in the marine subseafloor. Efforts to recruit microbiologists should therefore be emphasized. In order to reach this goal it is necessary to:

- Firmly establish that microbiologists working within IODP operate within the same general guidelines as the larger community of microbiologists with respect to common practices. (e.g. sequence submission, culture collections etc.).

- Expand scope of biological research in IODP by incorporating fields not traditionally related to ocean drilling (e.g. biotechnology, evolutionary science, bioremediation, astrobiology etc.).

- Sponsor sessions on ocean drilling at international microbiology meetings

- Establish a microbiological core repository for post-expedition sampling

6. Routine Measurements

A great strength of the scientific drilling program is the database of routine measurements that is openly accessible. This allows for continued analysis of the data using whether it is using new techniques or global syntheses of data (e.g. Parkes, *et al*., 2000; D'Hondt, *et al*., 2002). Therefore, it is necessary to institute routine measurements that can be realistically obtained during IODP drilling projects and provide useful data to assist in the study of subsurface microbiology.

a. Biomass. There are many methods for determining biomass, each with strengths and weaknesses. After comparing the methods on samples from cores, one should be instituted as a routine measurement. The possible candidates are:

i. *Direct cell counts*. By far, the largest microbiological dataset is biomass estimated by direct cell counts of microorganisms fluorescently labeled with acridine orange (Fry, 1988). Newer fluorochromes (e.g. SYBR Green) and flow cytometry should be examined for use within the program.

ii. *Vital stains*. There are several reagents available that indicate the level of metabolic activity by generating a fluorescent product (e.g. 5-cyano-2,3-ditolyl tetrazolium chloride; Proctor and Souza, 2001) that have been applied to sediments.

iii. *Phospholipids*. Intact phospholipids can be used to estimate the total microbial biomass in sediment samples (White*, et al.*, 1979; Zink*, et al.*, 2003).

iv. *ATP*. Adenosine-5'-triphosphate if found in a relatively constant proportion in all living cells. Quantification of this molecule to estimate total biomass has been used successfully in cores (Egeberg, 2000).

b. Metabolic Rates. The addition of the radioisotope isolation van into the program greatly extends the capabilities of the microbiologists. Because these measurements should be considered in the category of 'ephemeral properties' they must be initiated on board. While labor intensive, measurements that yield rates of metabolic processes (e.g. sulfate reduction, anaerobic methane oxidation, methanogensis, DNA and protein synthesis) can substantially change our view of the activities of microorganisms in the marine subsurface. These facilities should be available and the assays should be encouraged.

7) Additional Assays

a. Nucleic Acids. The analysis of nucleic acids has matured to the point where they can become routine. Initially, work has been focused on genes useful for phylogenetic analysis (e.g. small subunit ribosomal RNA), it has now expanded to include metabolic genes (e.g. dissimilatory sulfite reductase (dsr), Teske*, et al.*, 2003). These analyses can be conducted in shore-based laboratories so emphasis should be placed on routinely collecting and preserving samples on board the drilling platforms to later analysis.

b. Biomarkers. Similar to nucleic acid analysis, lipid biomarkers, especially when coupled to stable isotope analysis (e.g. Hinrichs*, et al.*, 1999) are extremely useful for characterizing the subsurface community. Samples for these analyses should be routinely collected onboard and preserved for shore-based analysis.

Recommendation 4. IODP institute a routine measurement program that will be performed in support of an ongoing study of microorganisms in the marine subsurface. The data produced from these assays will be submitted to the general

IODP database and be subject to the same stipulations as other data. IODP should routinely sail a technician dedicated to the microbiology laboratory. This technician will be responsible for training sailing microbiologists in the sampling procedures and sample analysis, maintaining the equipment in the microbiology laboratory, and ensuring that an adequate inventory of supplies are on hand prior to sailing. The technician should be specifically trained in microbiological techniques and procedures, including the use of radioisotopes, for the microbiology laboratory.

Summary

Through the efforts of the Ocean Drilling Program, much has been learned about microorganisms inhabiting the marine subsurface. In order to capitalize on this knowledge and advance the field during the Integrated Ocean Drilling Program, this working group provides the following recommendations.

Recommendation 1: IODP should establish a repository for samples routinely collected and stored appropriately for subsequent microbiological analysis. The samples should be taken in sterile syringes (50 cm^3 capacity) as soon as the core arrives and stored as described below depending on the subsequent analysis.

a. Samples for nucleic acid analysis should be placed immediately in liquid nitrogen and transferred to ultra-low freezer or liquid nitrogen on board for storage. Alternatively, whole round samples used for this purpose should be placed directly in an ultra-low freezer or liquid nitrogen as soon a possible. Because these samples are not useful for nucleic acid analysis after long term storage (> 1 year) they should be made available for other types of analyses (e.g. chemical) if appropriate.

b. Samples taken for culturing work should be transferred to gas-tight trilaminate bags containing an oxygen scrubber, heat-sealed and stored at 4 °C.

c. Samples for microscopy should be preserved with an aldehyde solution (electron microscopy grade glutaraldehyde or paraformaldehyde) and stored at 4 °C.

Recommendation 2: Drilling methods that yield cores of optimal quality for microbiological studies should become standard.

a. Routine use of the drill over method extends the useful range of the APC method and provides superior results for microbiological studies and should be implemented.

b. The continued development of the pressure retaining core barrel, and

subsequent handing under in situ pressures is highly valuable to the microbiology research and must be given highest priority.

c. Optimization of core processing with the goal of minimizing increases in temperature and exposure to oxygen should be implemented.

d. Continued performance, and further improvements to the methods for contamination testing (House*, et al.*, 2003) while coring.

Recommendation 3: IODP should adopt similar policies that are established within the international community of microbiologists for the exchange of culture and sequence data

a. Unique nucleic acid sequence data derived from cores and published in IODP publications or scientific journals must be submitted to one of the internationally recognized, publicly accessible databases (e.g. DDBJ, EMBL and GenBank).

b. Subcultures of organisms derived from cores and published in IODP publications or scientific journals must be deposited in at least two internationally recognized, publicly accessible culture collections (e.g. ATCC, JCM, DSMZ, and CCUG).

Recommendation 4. IODP institute routine measurements that will be performed in support of an ongoing study of microorganisms in the marine subsurface. The data produced from these assays will be submitted to the general IODP database and be subject to the same stipulations as other data. IODP should routinely sail a technician in the microbiology laboratory. This technician will be responsible for training sailing microbiologists in the sampling procedures and sample analysis, maintaining the equipment in the microbiology laboratory, and ensuring that an adequate inventory of supplies are on hand prior to sailing. The technician should be specifically trained in microbiological techniques and procedures, including the use of radioisotopes, for the microbiology laboratory.

References

Cragg, BA, Bale, SJ and Parkes, RJ. 1992. A novel method for the transport and long-term storage of cultures and samples in an anaerobic atmosphere. Letters in Applied Microbiology 15: 125-128.

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

Egeberg, PK. 2000. Adenosine 5'-triphosphate (ATP) as a proxy for bacterial numbers in deep-sea sediments and correlation with geochemical parameters (Site 994). Proceedings of the Ocean Drilling Program, Scientific Results 164: 393-398.

Fry, JC. 1988. Determination of biomass. in: Methods in Aquatic Bacteriology. B. Austin (ed). Wiley, Chichester, 27-72.

Hinrichs, K-U, Hayes, JÂ, Sylva, SP, Brewer, PG and DeLong, EF. 1999. Methane-consuming archaebacteria in marine sediments. Nature 398: 802-805.

House, CH, Cragg, B, A, and Teske, A. 2003. Drilling Contamination tests during ODP Leg 201 using chemical and particulate tracers. in: Proceedings of the Ocean Drilling Program, Initial Reports. D'Hondt, SL, Jørgensen, BB, and Miller, DJ (ed).

Parkes, RJ, Cragg, BA and Wellsbury, P. 2000. Recent studies on bacterial populations and processes in subseafloor sediments: A review. Hydrogeology Journal 8: 160.

Proctor, LM and Souza, AC. 2001. Method for enumeration of 5-cyano-2,3-ditoyl tetrazolium chloride (CTC)-active cells and cell-specific CTC activity of benthic bacteria in riverine, estuarine and coastal sediments. Journal of Microbiological Methods 43: 213-222.

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

Smith, DC, Spivack, AJ, Fisk, MR, Haveman, SA, Staudigel, H and Party, OLSS. 2000. Methods for quantifying potential microbial contamination during deep ocean coring. Ocean Drilling Program Technical Note 28 1-19.

Takai, K, Kobayashi, H, Nealson, KH and Horikoshi, K. 2003. Sulfurihydrogenibium subterraneum gen. nov., sp nov., from a subsurface hot aquifer. International Journal of Systematic and Evolutionary Microbiology 53: 823-827.

Teske, A, Dhillon, A and Sogin, ML. 2003. Genomic markers of ancient anaerobic microbial pathways: Sulfate reduction, methanogenesis, and methane oxidation. Biological Bulletin 204: 186-191.

White, DC, Davis, WM, Nickels, JS, King, JD and Bobbie, RJ. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Ocecologia 40: 51-62.

Zink, K-G, Wilkes, H, Disko, U, Elvert, M and Horsfield, B. 2003. Intact phospholipids--microbial "life markers" in marine deep subsurface sediments. Organic Geochemistry 34: 755-769.

[1]Journal statements on submission of sequence data:

FEMS Microbiology Ecology
Nucleotide sequences should be fully determined in both senses of the DNA. Sequence information will be accepted for publication only if: (a) it is relevant to a question of more general interest, (b) there is additional, complementary information, or (c) there is some particular, explicit reason for publication. All nucleotide and amino acid sequences must be deposited in an appropriate data bank. An accession number must be obtained before submission to the Editors and this fact should be mentioned in the covering letter. Authors are encouraged to use the EMBL Data Library but can also use other archives, such as GenBank. Authors should include the accession number in the appropriate Figure legend.

Applied Environmental Microbiology
It is expected that newly determined nucleotide and/or amino acid sequence data will be deposited and GenBank/EMBL/DDBJ accession numbers will be included in the manuscript no later than the modification stage of the review process. It is also expected that the sequence data will be released to the public no later than the publication date of the article. The accession number should be included in a separate paragraph at the end of the Materials and Methods section for long-form papers or at the end of the text for short-form papers. If conclusions in a manuscript are based on the analysis of sequences and a GenBank/EMBL/DDBJ accession number is not provided at the time of the review, authors may be required to provide the sequence data as a file on a floppy disk.
It is expected that when previously published sequence accession numbers are cited in a manuscript, the original citations (e.g., journal articles) will be included in the References section when possible or reasonable. Authors are also expected to do elementary searches and comparisons of nucleotide and amino acid sequences against the sequences in standard databases (e.g., GenBank) immediately before manuscripts are submitted and again at the proof stage.

[2]Journal statements on deposition of cultures in culture collections:

FEMS Microbiology Ecology. The editors expect that new and variant organisms, viruses and vectors described in FEMS journals will be made available, under written request and for their own use, to all qualified members of the scientific community. If delays in strain or vector distribution are anticipated or if they are available from sources other than the authors this should be indicated. The Editors encourage authors to deposit important strains in publicly accessible culture collections and to refer to the collections and strain numbers in the text. In the case of materials that have been distributed by individuals, authors should indicate the laboratory strain designations and name and address of the donor as well as the original culture collection identification number, if any.

Applied Environmental Microbiology. AEM encourages authors to deposit important strains in publicly accessible culture collections and to refer to the collections and strain numbers in the text. Since the authenticity of subcultures of culture collection specimens that are distributed by individuals cannot be ensured, authors should indicate laboratory strain designations and donor sources as well as original culture collection identification numbers.