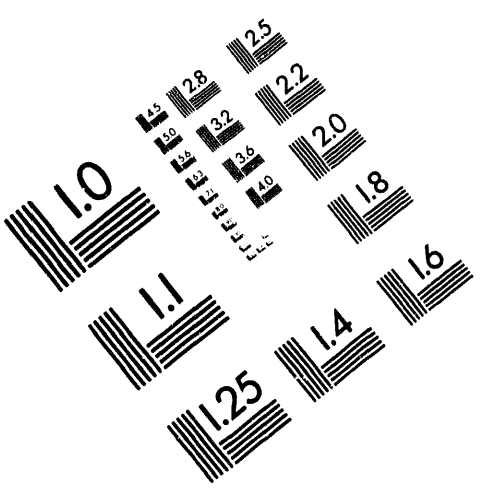
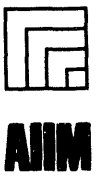
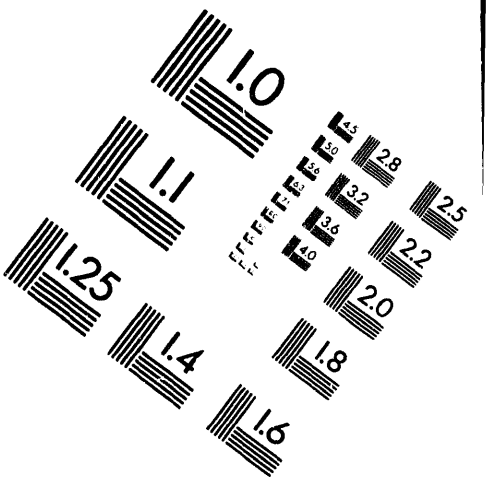
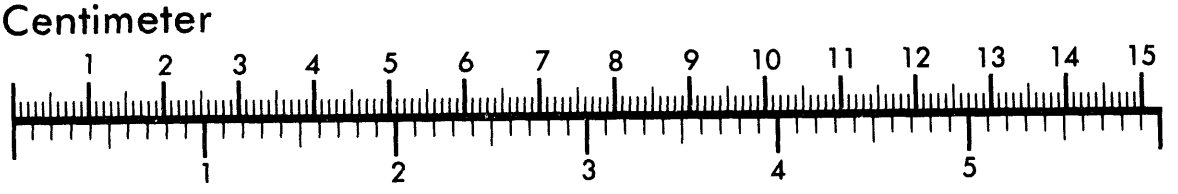
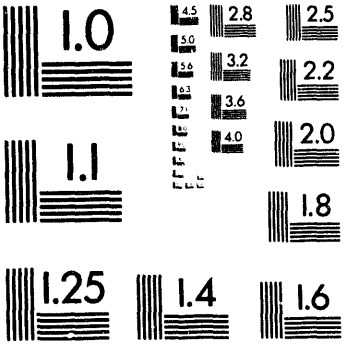


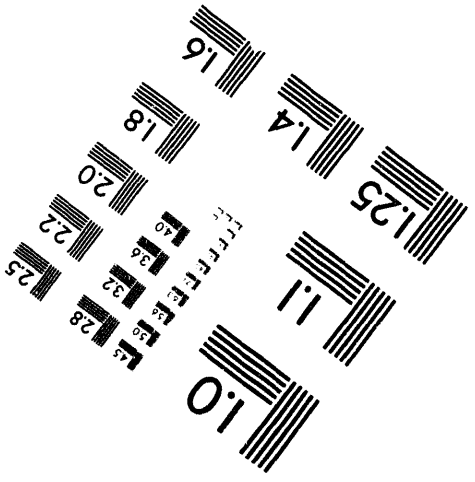


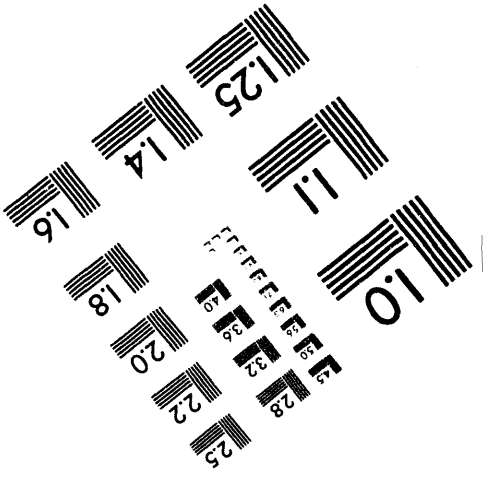
Association for Information and Image Management
1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202











1 of 1

DOE/ER/61039—2

THE DOE SUBSURFACE MICROBIAL CULTURE COLLECTION AT FLORIDA STATE UNIVERSITY

Interim Technical Report

August 15, 1993-March 15, 1994

David L. Balkwill, PI

Department of Biological Science

Florida State University

Tallahassee, Florida 32306

March 15, 1994

PREPARED FOR THE U. S. DEPARTMENT OF ENERGY
UNDER GRANT NUMBER DE-FG05-90ER61039

INTERIM TECHNICAL REPORT
FOR DOE RESEARCH GRANT NO. DE-FG05-90ER61039

B. Introduction

The U. S. Dept. of Energy (DOE) awarded a grant entitled "The DOE Subsurface Microbial Culture Collection at Florida State University" to Florida State University (D. L. Balkwill, PI) on August 15, 1993. This grant was actually a renewal of a project that had been funded previously (DOE Grant DE-FG05-90ER61039) to support research in the Deep Microbiology Subprogram of the Subsurface Science Program, by maintaining a culture collection of microorganisms isolated from subsurface environments (SMCC). The renewal grant was assigned a project duration of three years and funded for an initial budget period of one year (August 15, 1993 to August 14, 1994).

The present document (DOE Report No. DOE/ER/61039–2) is an interim technical report for the aforementioned renewal grant, in which we describe the research that has been completed during the seven-month period between the start of the project (August 15, 1993) and the date of this report (March 15, 1994). In accordance with DOE regulations, this report was prepared and is being submitted in support of a request for continued funding for the second year of the renewal project (August 15, 1994–August 14, 1995). The topical headings below correspond to the headings in the experimental plan of our original proposal (a copy of which is provided in Appendix I).

B. General Distribution of SMCC Cultures and Data Bases

1. Introduction

The general distribution of cultures and data (*i.e.*, distribution to scientists who are not involved in the Subsurface Science Program) was identified as an important function of the SMCC in 1991. We describe below our accomplishments related to this function of the culture collection during the period covered by this report.

2. Distribution of Cultures and Data Bases on Request

We did not distribute any cultures to non-DOE investigators during the first seven months of the project. (No cultures were requested.) However, we prepared various types of data base summaries (describing selected cultures in the collection) for 14 non-DOE scientists who requested information in response to articles describing the SMCC and its services (see below).

3. Development of a Representative Subset of Cultures

We proposed to develop several representative subsets of cultures in the SMCC because many potential users of the collection expressed strong interest in them. (By studying a representative subset of strains, they can readily determine how useful the full collection might be for their specific applications.) One such subset, consisting of approximately 75 strains from borehole P24 at the Savannah River Site, was developed during the period covered by this report. The cultures in this subset were chosen on the basis of preexisting 16S ribosomal RNA gene sequence analysis data (generated in our laboratory under separate funding) and data from the use of 16S ribosomal RNA-targeted hybridization probes (provided by Dr. S. Nierzwicki-Bauer at Rensselaer Polytechnic Institute).

4. Upgrading of Published SMCC Data Bases

Data bases describing the cultures in the SMCC are made available to users and potential users of the collection on request. These data bases were upgraded during the first seven months

of the project in two ways: (i) by adding new data on the phylogenetic characteristics (16S rRNA gene sequence data and 16S rRNA-targeted hybridization probe data) of strains isolated from the P24 and P29 boreholes at the Savannah River Site, and (ii) by developing a specialized data base describing the first representative subset of strains in the SMCC (see above).

5. Support of Collaborative Technology Transfer Efforts

Throughout the period covered by this interim report, we collaborated with officials in the Environmental Science Research Center (ESRC) and Pacific Northwest Laboratory (PNL) on the development of a CRADA to promote commercial use of the SMCC and its services. No current project funds were expended for this collaboration; it is mentioned here only to provide the reader with a full picture of our efforts to encourage technology transfer.

6. Promotion of the SMCC and Its Services

Articles describing the SMCC and its services were published in the *United States Federation of Culture Collections Newsletter* and in *ASM News* during the period covered by this report. Copies of these articles are provided in Appendix II. The availability of cultures and services was also announced in *Groundwater Microbiology Newsletter*.

C. Continuation of the SMCC's Regular Services to DOE

1. Introduction

The most important function of the SMCC is to provide various culture-related services to DOE and DOE-funded investigators on request. The main purpose of these services is to support research in the Subsurface Science Program, especially Transitional Phase and Phase II (Origins Initiative) of the Deep Microbiology Subprogram. We describe below the services that were provided during the period covered by this report.

2. Production of Standard Culture Sets for Phase II (Origins) Research

Much effort was devoted to the development of "focus clades" and other groups of strains for use by PIs in the Phase II-Origins Initiative of the Deep Microbiology Subprogram during the first seven months of the project. Detailed descriptions of culture groups that were likely to be of interest were prepared and presented to the Origins PIs at the first Origins Investigators' Meeting in December, 1993. Discussions were held with all new Origins PIs, in order to better define how the SMCC could meet their specific research needs. Based on these discussion, additional culture descriptions were prepared and additional analyses (primarily 16S ribosomal RNA gene sequence analyses) were performed on potential "focus clade" strains.

3. Distribution of Cultures and Data on Request

We distributed a total of approximately 675 cultures to seven Deep Microbiology Subprogram investigators during the first seven months of the project. About 25% of the requested cultures were used in research associated with the Transitional Phase of the program; the other 75% were used in research associated with the Phase II-Origins initiative. The investigators were also provided with data summaries describing the cultures and the environments from which they were isolated.

4. Incorporation of Newly Isolated Microbial Strains

Approximately 2,400 new subsurface microbial isolates were incorporated into the SMCC during the period covered by this report. Four hundred of the new isolates were associated with

the GEMHEX experiment of the Transitional Phase program and came from the YB-02 borehole at the Hanford Site. The other 2,000 new isolates came from several vadose-zone samples taken at the Hanford Site for a preliminary study on transport of microorganisms in the subsurface (Dr. Ellyn Murphy, PI).

5. Preservation of Newly Incorporated Strains

All of new isolates described above were preserved in genetically stable form as specified in the original proposal during the period covered by this report.

6. Partial Characterization of Newly Incorporated Strains

Colony morphological characteristics (size, color, surface type, elevation, etc.) were determined and recorded for each of the 2,400 newly incorporated strains described above during the first seven months of the project. Cell morphological characteristics (Gram reaction, shape, size, etc.) were determined for 1,100 of the new isolates, and 21 selected physiological traits (measured with the API Rapid NFT system, as described in the original proposal) were determined for 2,200 of the new isolates.

7. Phylogenetic Analysis of Selected SMCC Strains

One new isolate from the Texaco borehole (exploratory Origins research) was characterized by analysis of 16S ribosomal RNA gene nucleotide base sequences during the period covered by this report. This isolate (TH-23) was obtained by Dr. D. Boone at Oregon Graduate Institute, and was found to be a new species of *Bacillus*.

8. Continued Maintenance of SMCC Data Bases

All of the morphological and physiological characteristics determined for newly incorporated strains (see above) were added to the SMCC culture data base during the period covered by this report. Newly obtained phylogenetic information on previously incorporated strains was also added to the culture data base during the reporting period.

D. Continued Quality Assurance/Quality Control (QA/QC) Activities

The SMCC's standard QA/QC activities were continued throughout the first seven months of the project, as described in the original proposal (Appendix I).

Appendix I

Copy of Experimental Plan from the Original Proposal for this Project

EXPERIMENTAL PLAN

A. General Distribution of SMCC Cultures and Data Bases

1. Introduction

We will continue to provide cultures and descriptive data to non-DOE investigators on request during the three-year period for which new funds are sought. At the same time, we plan to enhance the SMCC's utility and accessibility to non-DOE users by: (i) developing and offering a representative subset of isolates in the collection, (ii) upgrading the data bases that are made available to potential users of the collection, (iii) supporting collaborative technology transfer initiatives, and (iv) publicly promoting the SMCC and its services. These enhancement activities and some of the mechanisms by which cultures might be distributed are described in more detail below.

2. Distribution of Cultures and Data Bases on Request

We will continue to distribute cultures and data to investigators at nonprofit institutions under the arrangement that was established during the first year of our current grant. Those who request cultures under this arrangement will have to affirm that they are qualified and equipped to handle uncharacterized (*i.e.*, potentially pathogenic) microorganisms, but they will not be required to specify how they intend to use them.

We will also distribute cultures and descriptive data to interested parties at commercial (*i.e.*, for-profit) organizations. In such cases, the cultures will be provided either under the arrangement used for nonprofit institutions (above) or under any of several alternative mechanisms that will probably arise as we collaborate with officials at PNL on the development of CRADAs and other strategies for technology transfer (below). The exact terms of these alternative mechanisms have not yet been defined.

Users of the SMCC (including nonprofit institutions, commercial organizations, and private individuals) will not be granted ownership of or exclusive rights to the cultures they receive. Rather, cultures will be supplied through a bailment agreement in which the recipient essentially leases them for a limited period of time. The recipients will have to agree not to pass the cultures on to third parties without written permission. Except for this prohibition, recipients will have unrestricted use of the cultures while the bailment is in effect. At the same time, the cultures will remain available to anyone else who wants to work with them (including the PI and his co-workers). Culture recipients may be able to claim full or partial ownership of any inventions or patents arising from their research with the cultures, depending on the mechanism under which their bailment is established.

We expect (based on our experience to date) to receive occasional requests for very small numbers of cultures (sometimes for only a single culture) from scientists at nonprofit institutions. We plan to handle such requests informally by waiving the SMCC's usual fees to cover transfer and shipping costs. Specifically, we will supply up to ten cultures per year without charge to any individual or working group at any kind of nonprofit institution (university, government agency, etc.). The rationale for this policy is twofold: (i) most of these requests are likely to come from unfunded investigators who cannot purchase cultures with institutional funds, and (ii) it would be uneconomical to process the paperwork required to handle these small requests formally. The cost of maintaining a free culture service should be minimal and will be incorporated into the collection's regular operating budget.

Most requests for cultures from the SMCC are likely to involve larger quantities of isolates or to come from commercial (*i.e.*, for-profit) organizations. In these cases, the cost of reproducing and shipping the cultures must be borne by those who request them. Therefore, orders for more than ten cultures (per year per investigator or working group; above) and *all* orders from commercial organizations will be subjected to fees according to the following schedule:

1-9 cultures	$ 45.00 per culture
10-49 cultures	$ 40.00 per culture
50-99 cultures	$ 35.00 per culture
100-499 cultures	$ 30.00 per culture
500-999 cultures	$ 25.00 per culture
1000 or more cultures	$ 20.00 per culture

The above charges represent our best estimate of the actual cost of reproducing the cultures, performing QA/QC procedures (*e.g.*, checking for viability and purity), and shipping (including standard university indirect costs). The specified quantity discounts reflect the fact that it is considerably more efficient to reproduce large groups of cultures simultaneously than it is to reproduce small groups of cultures or single isolates. The fees include a data base printout for each culture acquired (listing everything known about the cultures at the time) and a replacement culture if the copy supplied originally fails to grow.

Organizations who wish to acquire SMCC cultures in accordance with the above fee schedule will have to: (i) sign a hold-harmless agreement exempting FSU from any liabilty problems arising from the recipient's use of the cultures, (ii) sign an agreement not to provide cultures to third parties without permission, and (iii) issue a purchase order to FSU in the appropriate amount. The cultures will then be provided in a timely manner.

3. Development of a Representative Subset of Cultures

Many of the potential SMCC users who have contacted us about acquiring cultures under our current distribution mechanism have wanted to know whether they can obtain a representative subset of the strains in the collection. (This question is most often asked by potential users associated with commercial organizations.) The interest in a subset of cultures is generally based on either of two concerns: (i) the potential user wishes to perform an expensive screening procedure on the cultures and they are worried that a large amount of money will be wasted on duplicate (or nearly duplicate) strains if they look at the whole collection, or (ii) the potential user cannot afford a large number of cultures but still wants to look at a fairly representative sampling of the types that may be present. In any case, it is clear that having the ability to offer one or more representative subsets of isolates would significantly enhance the SMCC's perceived value among its potential users. This, in turn, should lead to more widespread use of the cultures among non-DOE investigators.

Until recently, it was impossible to do anything about the need for a representative subset of strains because we knew next to nothing about the possible identity (or even general taxonomic position) of the cultures in the SMCC. However, we are currently carrying out a phylogenetic characterization of strains from the SRS (under separate DOE funding; DOE Grant No. DE-FG05-91ER61159), in which we are making use of molecular biological approaches like 16S ribosomal DNA sequencing and restriction fragment length polymorphism analysis. This effort is coordinated with an investigation directed by Dr. Sandra A. Nierzwicki-Bauer at Rensselaer Polytechnic Institute (RPI) that is based on group- and species-specific, 16S rRNA-targeted, oligonucleotide hybridization probes. The results obtained at FSU and RPI so far would allow us to select a subset of approximately 85 isolates

from SRS borehole P24 that: (i) is representative (phylogenetically) of the entire group of about 700 cultures that were isolated from P24 samples, and (ii) does not include duplicate or near-duplicate strains.

We propose to utilize the aforementioned data, along with the SMCC's QA/QC records (*e.g.*, purity or viability checks and notes on how readily the cultures have transferred in the past) to select the most useful isolates for inclusion in a representative subset of P24 strains. As the ongoing phylogenetic characterization research produces the required data (under separate funding), we will designate similar subsets of the isolates from other boreholes at the SRS and from boreholes at other DOE sites (*e.g.*, INEL, HR, and/or NTS). If enough data become available, we will eventually identify a somewhat larger subset of cultures that is representative of the entire collection.

Each time a new representative subset of isolates is selected, we will prepare a written description of the subset that summarizes the known characteristics of the cultures in it and explains how they were chosen. These descriptions will be made available to potential users of the SMCC, and their existence will be featured in efforts to promote the collection and its services (below). If there is enough demand for them, we will make up extra stocks of the more popular subsets in advance (to facilitate rapid handling of requests for them).

4. Upgrading of Published SMCC Data Bases

When DOE originally decided to make the cultures and services of the SMCC available to the general scientific community, we realized that many scientists would need some means for determining which of the more than 4,500 strains in the collection (at that time) were suitable for their specific purposes. (Otherwise, the collection and its services would be of only limited use to most researchers.) As a result, we decided to develop "published" versions of the SMCC data bases (the Culture Data Base and Sample Data Base) that: (i) contained any information about the cultures that had been released for publication by the Deep Microbiology Program PIs, and (ii) could readily be distributed to potential users of the SMCC on request. Several alternative formats were considered, after which we decided that magnetic versions of the data bases (*i.e.*, digitized versions on magnetic media such as floppy disks) would be most useful. Unlike printed documents, magnetic versions of the data bases can be updated frequently (at very low cost) and can be searched automatically with various data base management programs. We can now provide the data bases in several formats (for different types of computers and data base programs), so that almost any potential SMCC user can quickly locate the types of strains they need.

During the three-year period for which new funds are requested, we propose to upgrade the published data bases that are made available to potential users of the collection in three ways: (i) by adding new information on the phylogenetic characteristics of the cultures (including their possible identity), (ii) by producing specialized data bases to describe the cultures that are included in the representative subsets of isolates (see above), and (iii) by instituting electronic mail ("E-mail") access to the data bases (thus eliminating the need to send disks through the mail). These improvements will significantly enhance the usefulness of the published data bases and, thereby, help to promote more widespread use of the collection itself.

5. Support of Collaborative Technology Transfer Efforts

We will continue to collaborate with scientists and officials at PNL on the development of CRADAs and other technology transfer agreements, to foster increased use of the SMCC and its services by the private sector (especially within the biotechnology industry).

This collaboration was initiated quite recently, so cannot say exactly how we intend to proceed with it at this time. (We are still trying to find out which alternatives are feasible). In any case, we are not requesting funds for the PNL collaboration in this proposal. (It would be inconsistent with the regulations under which the CRADA system was established to do so.) We mention the collaboration here simply to provide the reader with a complete picture of our efforts to encourage more widespread use of the SMCC.

6. Promotion of the SMCC and Its Services

The cultures and services of the SMCC will be utilized only if the scientific community is fully aware of their existence. Consequently, we will continue to promote the collection publicly in the following ways:

- We will prepare brief news articles describing the current status of the collection and its services (as well as the procedures for acquiring cultures). These will be submitted to prominent microbiology newsletters (such as *ASM News* and *SIM News*) and other publications that are likely to be scanned by potential users of the SMCC.

- Whenever we make noteworthy improvements in the SMCC and its services (as a result of the funding sought in this proposal), we will prepare announcements describing these improvements and send them to appropriate newsletters or other publications.

- We will present information on the SMCC and its services at scientific meetings and symposia whenever possible.

B. Continuation of the SMCC's Regular Services to DOE

1. Introduction

We are submitting this proposal not only to facilitate the general distribution of cultures in the SMCC to interested scientists, but also to ensure a continuation of the SMCC's regular services to DOE and DOE investigators. The reasons for continuing these services were detailed in the Introduction and Background (above). Following is a summary of the specific services that we propose to provide during the three-year period for which funding is requested.

2. Production of Standard Culture Sets for Phase II (Origins) Research

It was strongly recommended at a recent DOE-sponsored workshop (the Molecular "Clocks" Workshop; Sept. 30-Oct. 2, 1992, in Annapolis, MD) that all PIs who wish to study the possible origins of microorganisms in the subsurface (in Phase II of the Deep Microbiology Program) by examining pure cultures should focus on selected groups of very closely related cultures (referred to as "clades") in the SMCC. Among the different groups of isolates recommended for Phase II research were those related to *Acinetobacter calcoaceticus, Comamonas testosteroni* (formerly *Pseudomonas testosteroni*), *Arthrobacter globiformis*, and *Pseudomonas aeruginosa.*

The specific isolates to be included in clades for Phase II research will be identified under separate funding (at FSU and RPI). Once these strains have been chosen, however, it will be the responsibility of the SMCC to make sure that they are readily available to all Phase II investigators who need them. It is extremely important that investigators working

with a particular clade all receive exact duplicates of the cultures in that clade. Therefore, we will grow up a single broth culture of each clade strain and then prepare replicate frozen stocks from this single culture. The frozen stocks will be prepared in the usual way (see above), except that we will make 50 duplicate vials from each broth culture. This plan will ensure that all interested PIs can obtain a reliable set of cultures, as well as obtain replacements for any cultures that are accidentally lost. The frozen stocks will be sent to the program PIs on dry ice, so that they can be reactivated in each investigator's laboratory.

3. Distribution of Cultures and Data on Request

We will continue to provide SMCC cultures and information to DOE-funded investigators on request. To obtain cultures, PIs need only to give us a list of the desired strains and tell us when they would like to receive them. We will then ship the cultures as soon as they have been successfully activated and transferred (usually within a few days). Cultures will be supplied on slants unless other arrangements are made in advance. The strains will normally be provided to DOE investigators without charge (a service supported by funding requested in this proposal). However, investigators who need more than 1000 cultures per year will be expected to request funding of their own to help cover the expense of growing and shipping such large numbers of strains.

DOE investigators can also obtain data or complete copies of the SMCC data bases without charge, by simply contacting our laboratory and telling us what they need. We will then supply the requested information in the form of printed reports ("hard copies"), magnetic copies, or (once it has been installed) by electronic mail service.

4. Incorporation of Newly Isolated Microbial Strains

We will continue to incorporate new strains of subsurface microorganisms obtained by DOE investigators throughout the three-year period for which new funds are requested. Some new strains could be isolated during extended Transitional Phase activities (*e.g.*, during additional GEMHEX-type projects), but most of them are likely to be produced during Phase II research focusing on the origins of subsurface microorganisms (7). It is not possible to predict the exact number of strains that will be isolated during Phase II, but our past experience indicates that we can expect as many as 500 new isolates each year (depending on the number of samples examined). In any case, the incorporation of new strains will be performed as follows:

- We will first assign a permanent accession number to each new isolate. This number serves as a master reference that may be used to rapidly identify and describe each strain in the future.

- We will enter information on the subsurface sample from which each culture was isolated into the SMCC Sample Data Base. This data base allows investigators to search the collection for microbes that were isolated from specific kinds of subsurface environments. The information entered for each sample includes (but is not necessarily limited to): (i) the geographic location of the sampling site, (ii) the depth and geological formation from which the sample was obtained, (iii) all known chemical characteristics of the sample (*e.g.*, pH, DOC, TOC, and concentrations of inorganic nutrients), (iv) all known physical characteristics of the sample (*e.g.*, grain size, porosity, and hydraulic conductivity), and (v) a geological description of the sample.

- We will enter information on each new culture into the SMCC Culture Data Base. This data base can be used to rapidly search the collection for isolates with specific traits. The information entered for each isolate includes (but is not necessarily limited to): (i) origin (laboratory that obtained it and subsurface sample from which it was isolated), (ii) cultural characteristics (optimal incubation temperature, nutrient medium, etc.), (iii) cell and colony morphological properties, (iv) physiological traits (primarily responses to API Rapid NFT and other semi-automated testing systems), and, if known, (v) phylogenetic and taxonomic characteristics (based on molecular biological and other analytical methods).

5. Preservation of Newly Incorporated Strains

Each isolate incorporated into the SMCC during the three-year period for which we are requesting new funds will initially be stabilized (to prevent genetic changes) as follows. Strains growing on the original enumeration plates (which are used for the determination of viable cell counts in our laboratory) or submitted by other DOE researchers will be purified by streak plating on fresh medium. The number of transfers on laboratory media will be kept to the minimum required to ensure purification of the culture. Purified strains will then be grown to mid-exponential phase in broth media (liquid version of the original isolation medium), concentrated by low-speed centrifugation, resuspended in a small volume of fresh medium containing 7% sterile dimethylsulfoxide (DMSO), and frozen at -75°C. Two stocks of each culture will be prepared in this way. One (the working stock, from which we will prepare active cultures on request) will be stored at -75°C in a freezer equipped with a temperature recording chart, liquid-CO_2 backup system, and automatic alarm system. The second frozen stock (the master "insurance" stock) will be stored under liquid nitrogen in a secured (padlocked) dewar (-196°C).

We will continue to produce lyophilized (freeze-dried, preserved) stocks of cultures in the SMCC by employing the Speedvac approach that we developed a few years ago (11). Because this methodology is somewhat tedious and time-consuming, however, we will only produce new lyophilized stocks on a "when time and resources permit" basis. In any event, we will grant highest priority to the production of representative subsets of SMCC cultures for rapid distribution to DOE and non-DOE investigators on request (see above).

6. Partial Characterization of Newly Submitted Strains

We will partially characterize each new subsurface microbial isolate that is incorporated into the SMCC during the period for which new funding is sought. In most cases, the characterization will include documentation of the following traits:

- Cultural traits (preferred growth medium, incubation conditions and temperature, growth rate, etc.).
- Cell morphological traits (shape, size, tendency to form pairs or other groupings, reaction to Gram stain, presence of intracellular inclusions, etc.).
- Colony morphological traits (relative size, color, elevation, type of edge, surface features, opacity, etc.).
- Physiological traits (reactions to standard testing kits, such as the API Rapid NFT system or the Biolog system, depending on the needs of the investigator who submitted the culture to the collection).

All of the data from partial characterization of new cultures will be entered into the SMCC Culture Data Base, thus enhancing the usefulness of the newly incorporated strains to researchers in the future.

7. Phylogenetic Analysis of Selected SMCC Strains

We propose to determine the phylogenetic characteristics of selected SMCC strains at the request of program PIs during the three-year period for which new funds are sought. We are currently examining the phylogenetic traits of certain groups of cultures in the collection under separate DOE funding (DOE Grant No. DE-FG05-91ER61159), and we will continue to do this for at least another year. There have been several occasions during the past few months, however, in which Deep Microbiology Program PIs have urgently needed phylogenetic information on SMCC cultures that are not among the groups being analyzed under separate funding. We have examined these cultures for the PIs on an informal collaborative basis whenever possible (in keeping with the generally interactive structure of the program). Now that the demand for phylogenetic analyses has been established, we would like to include these analyses among the regular services of the SMCC. The methods used for phylogenetic characterization are quite expensive and time-consuming. As a result, the service would be provided only on a "first come, first served" and "when time and resources permit" basis. Our experience to date indicates that this level of service should adequately address the critical needs of the PIs in the program.

Phylogenetic characterizations performed under the circumstances described above will make use of one or more of the following techniques:

- Restriction fragment length polymorphism (RFLP) analysis, which can rapidly determine whether the strain in question is closely related (or identical to) any SMCC strains that have already been characterized phylogenetically.

- Analysis of 16S ribosomal DNA nucleotide base sequences, which will determine how the isolate in question is related to established microbial taxa (*i.e.*, will essentially identify the isolate).

- Use of species- or group-specific 16S rRNA-targeted oligonucleotide hybridization probes, which can assign the isolates in question to any of the species or groups for which the probes are currently available. (Hybridization probe analyses would be done in collaboration with RPI.)

All data stemming from the phylogenetic characterization of subsurface microorganisms will be entered into the SMCC Culture Data Base and, as a result, will be available to interested scientists in the future.

8. Continued Maintenance of SMCC Data Bases

We will continue to maintain and update the SMCC Sample Data Base and Culture Data Base throughout the period for which new funding is sought. The types of data which are stored in these data bases are described in the preceding sections. The data bases will also be subjected to the QA/QC measures described below.

C. Continued Quality Assurance/Quality Control (QA/QC) Activities

We will continue our standard QA/QC procedures throughout the period for which new funding is requested by reexamining 5% of the strains in the collection with respect to viability, culture purity, cell morphology, colony morphology, and physiological characteristics. The findings will then be compared to the information that is currently stored in both SMCC data bases, as well as to all descriptions and observations that were recorded as the strains were originally isolated and incorporated into the collection. Of the strains that are reexamined for QA/QC purposes, 50% will be from the SRS samples studied in Phase I of the Deep Microbiology Program and 50% will be from samples (from different DOE sites) examined during the Transitional Phase of the program.

As an added QA/QC measure, we will ask an independent investigator—Dr. James K. Fredrickson at PNL—to examine 100 cultures (over the three-year duration of the project) as "blind" samples. Dr. Fredrickson will describe the morphological and physiological traits of each strain (without knowing our own descriptions of these traits) and transmit his findings to us for comparison. Any discrepancies that are noted will then be discussed and resolved to the satisfaction of both investigators. We will supply all of the materials needed to carry out the analyses (including physiological testing kits) from well documented lot numbers.

The QA/QC measures described above will allow us to carry out a continuing evaluation of the SMCC data bases, an evaluation that, in turn, should enable us to ensure that these data bases remain accurate and useful to researchers in the future.

REFERENCES

1. U. S. Department of Energy. 1986. *Microbiology of Subsurface Environments—Preliminary Statement of Research Goals and Abstracts of Current Research*. DOE/ER-0278, Office of Health and Environmental Research, Office of Energy Research, U. S. Department of Energy, Washington, D. C.

2. Balkwill, D. L., and F. J. Wobber. 1989. *Transitional Program Plan—Research Plan for a Comparative Study of Deep Subsurface Microbial Communities at Department of Energy and Other National Sites*. DOE/ER-0328, Office of Health and Environmental Research, Office of Energy Research, U. S. Department of Energy, Washginton, D. C.\

3. Wobber, F. J. 1989. *Deep Microbiology Transitional Program—Implementation Plan*. DOE/ER-0431, Office of Health and Environmental Research, Office of Energy Research, U. S. Department of Energy, Washington, D. C.

4. Wobber, F. J., J. K. Fredrickson, and R. E. Wildung. 1989. *Microbiology of Subsurface Environments: Program Plan & Summary—Phase II*. DOE/ER-0293, Office of Health and Environmental Research, Office of Energy Research, U.S. Department of Energy, Washington, D. C.

5. U. S. Department of Energy. 1991. *Subsurface Science Program—Program Overview & Research Abstracts, FY 1989-FY 1990*. DOE/ER-0432, Office of Health and Environmental Research, Office of Energy Research, U. S. Department of Energy, Washington, D. C.

6. Long, P., S. Rawson, J. Fredrickson, and J. Zachara. 1992. *Deep Microbiology Transitional Phase GEMHEX Implementation Plan.* Battelle Pacific Northwest Laboratory, Environmental Science Research Center, Richland, Wash.

7. U. S. Department of Energy. 1992. *Origins of Microorganisms in Deep Subsurface Environments—Deep Microbiology Subprogram Phase II Preliminary Plan.* DOE/ER-0556T, Office of Health and Environmental Research, Office of Energy Research, U. S. Department of Energy, Washington, D. C.

8. Balkwill, D. L., J. K. Fredrickson, and J. M. Thomas. 1989. Vertical and horizontal variations in the physiological diversity of the aerobic chemoheterotrophic bacterial microflora in deep Southeast Coastal Plain subsurface sediments. *Appl. Environ. Microbiol.* 55:1058-1065.

9. Fredrickson, J. K., D. L. Balkwill, J. M Zachara, S.-M. Li, F. J. Brockman, and M. A. Simmons. 1991. Physiological diversity and distributions of heterotrophic bacteria in deep Cretaceous sediments of the Atlantic coastal plain. *Appl. Environ. Microbiol.* 57:402-411.

10. *Geomicrobiology Journal.* 1989. Special Issue on Deep Subsurface Microbiology, Vol. 7:(1-2).

11. Balkwill, D. L. 1990. Deep-aquifer microorganisms. *In:* D. P. Labeda (ed.), *Isolation of Biotechnological Organisms from Nature*, McGraw Hill Publishing Co., New York, pp. 183-212.

reprints returned.
ds

DATE
FILMED
9/16/94

END