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THE DOE SUBSURFACE MICROBIAL CULTURE COLLECTION (SMCC)

Final Technical Report

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FINAL TECHNICAL REPORT
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A. Introduction

The U.S. Department of Energy (DOE) awarded a grant entitled "The DOE Subsurface Microbial Culture Collection (SMCC)" to Florida State University (Dr. David L. Balkwill, PI) on August 15, 1996. This grant was funded to support research associated with the Natural and Accelerated Bioremediation (NABIR) Program. The primary objectives of the project were (1) to maintain and characterize a culture collection of microorganisms isolated from terrestrial subsurface environments, and (2) to carry out collaborative research with other PIs in NABIR and other DOE-funded research programs.

The aforementioned grant was assigned a project duration of three years (*i.e.*, August 15, 1996-Augsut 14, 1999) and funded throughout that period. The grant subsequently was renewed and funded for a second three-year project period (August 15, 1999-August 14, 2002). Another three-year renewal was awarded starting August 15, 2002. However, during the first year of this project, DOE decided that the culture collection was no longer essential for support of its future research directions. Consequently, the renewal grant was funded for a second and final year (*i.e.*, August 15, 2003-August 14, 2004). A no-cost extension was approved later, thereby making the final end date of the project August 14, 2005. This final technical report summarizes the activities supported by DOE throughout the entire duration of the project (August 15, 1996-August 14, 2005).

B. Provision of SMCC Cultures and Resources to DOE and Other Parties

1. Introduction

The primary activities associated with maintenance of the Subsurface Microbial Culture Collection (SMCC) were designed to ensure that the collection served as a valuable resource to DOE-funded and other scientists, especially DOE-funded scientists associated with the NABIR Program. These activities were carried out throughout the period covered by this report and included: (1) assistance in the selection of cultures for research, (2) distribution of cultures and/or data on request, (3) incorporation of newly isolated microbial strains, (4) preservation of newly isolated strains, (5) partial characterization of newly isolated strains, (6) development and maintenance of representative subsets of cultures, (6) screening of SMCC strains for specific characteristics, (7) phylogenetic characterization of SMCC strains, (8) development and maintenance of a SMCC website, (9) maintenance of the SMCC databases, (10) archiving of SMCC records, and (11) quality assurance/quality control (QA/QC) activities. We describe below our accomplishments related to these activities during the period covered by this report.

2. Assistance in the Selection of Cultures for Research

The PI and his staff searched the SMCC's extensive culture databases, analyzed SMCC's collection of 16S rRNA gene sequences, and checked relevant research records and publications, in order to help interested scientists find specific SMCC strains or sets of strains that might be of

use in their research programs. Approximately 65 DOE-funded and non-DOE-funded scientists were assisted in this way during the period covered by this report. Potentially useful strains were located within the collection in about 75% of these incidents.

3. Distribution of Cultures and Data on Request

More than 4,500 microbial cultures and associated data were provided to interested scientists during the period covered by this report. Approximately 90% of the cultures were provided to DOE-funded scientists. The DOE-funded scientists included those involved with the NABIR Program as well as non-NABIR scientists at some of the national laboratories. Among the DOE-funded scientists who received cultures were: T. Barkey (Rutgers Univ.), D. Boone (Oregon Graduate Univ.), F. Brockman (Pacific Northwest National Laboratory; PNNL), R. Crawford (Univ. of Idaho), M. DeFlaun (Envirogen), C. Fliermans (Savannah River Site), J. Fredrickson (PNNL), W. Holben (Univ. of Montana), T. Kieft (New Mexico Tech.), R. Miller (Oklahoma State Univ.), T. Phelps (Oak Ridge National Laboratory), M. Romine (PNNL), T. Schmidt (Michigan State Univ.), D. White (Univ. of Tennessee), and K. Wong (PNNL).

Non-DOE-affiliated scientists who were provided with cultures or culture-related data included: S. Bagley (Michigan State Univ.), C. Belanger (Fisheries and Oceans Canada), H.-J. Busse (Univ. of Helsinki), C. Case (Skyline College), A. Darzins (Energy Biosystems Corp.), D. Haag (Boehringer Ingelheim Co.), H. Hatano (Osaka Institute for Fermentation), D. Janssens (Univ. of Ghent), K. Johnsen (Geological Survey of Denmark), K. Kwon (Tuskegee Univ.), A. Laskin (Drew University), P. Lau (NRC Biotechnology Institute), J. Piggott (Zymogenetics, Inc.), F. Robb (Univ. of Maryland), A. Simon (Univ. of Massachusetts), and S. Welch (Univ. of Wisconsin). Cultures of SMCC strains that have been identified as novel species were also supplied as a courtesy to several other major culture collections on request, including the American Type Culture Collection, the Collection de Bacteries de l'Institut Pasteur (France), the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Germany), and the National Collection of Industrial and Marine Bacteria (England).

4. Incorporation of Newly Isolated SMCC Strains

Approximately 6,700 new subsurface bacterial isolates were incorporated into the SMCC during the period covered by this report. Each new strain was assigned a permanent accession number, and all available information on the sources of the new strains and the environments from which they were isolated was added to the SMCC databases (see below). The actual numbers of new strains incorporated and their sources were as follows:

- 257 strains from the Oyster site in Virginia (contributed by M. DeFlaun)
- 1,697 strains from the Oyster site in Virginia (contributed by D. Balkwill)
- 120 strains from the Cerro Negro site in new Mexico (contributed by L. Forney)
- 14 strains from the Sanpoil site at Hanford (contributed by F. Brockman)
- 44 strains from the Washtuchna site at Hanford (contributed by F. Brockman)
- 415 strains from the Abbott's Pit site in Virginia (contributed by D. Balkwill)
- 312 strains from deep South African gold mine rocks (contributed by T.C. Onstott)
- 290 strains from the Dover Air Base site in Delaware (contributed by D. Balkwill)

- 787 strains from the Cape Charles site in Virginia (contributed by D. Balkwill)
- 1,229 strains from the Shiprock UMTRA site (contributed by D. Balkwill)
- 1,199 strains from the Gunnison UMTRA site (contributed by D. Balkwill)
- 19 metal-resistant strains from various sties (contributed by A. Konopka)
- 81 strains from Columbia River sediments at Hanford (contributed by J. Fredrickson)
- 132 strains from SX-108 tank soil at Hanford (contributed by J. Fredrickson)
- 81 strains from the NABIR FRC site at ORNL (contributed by D. Balkwill)

5. Preservation of Newly Incorporated Strains

All of the newly incorporated strains listed above were preserved in a genetically stable form for possible future use by freezing in a cryoprotectant and subsequent storage at -80C (in a freezer) and at -196C (under liquid nitrogen).

6. Partial Characterization of Newly Incorporated Strains

Colony morphological characteristics (size, color, type of surface, elevation, etc.) were determined for each of the 6,700 newly incorporated strains described above during the period covered by this report. Cell morphological characteristics (cell shape and Gram stain reaction) were determined for approximately 1,400 strains (some of which were incorporated prior to the start of this project) during the period covered by this report.

7. Development and Maintenance of Representative Subsets of Cultures

Representative subsets of cultures from certain subsurface environments were developed and maintained at the request of DOE-funded scientists in the NABIR Program. The purpose of these subsets was to allow investigators to examine a fairly complete representative sampling of the different types of bacteria that were isolated from a particular environment, without having to waste resources on large numbers of closely related or otherwise very similar organisms. Strains for the subsets were selected on the basis of morphological characteristics, selected physiological traits, fatty acid profiles (MIDI analyses), and/or phylogenetic analysis of 16S rDNA sequences. Databases containing all known information on the strains in each subset were also prepared and made available to interested investigators. Three representative subsets of SMCC strains were developed during the period covered by this report, as follows:

- A set of 100 bacteria from deep Atlantic Coastal Plain aquifers at the Savannah River Site
- A set of 50 bacteria from saturated lacustrine sediments, paleosols, and fluvial sands at the Hanford Site
- A set of 120 bacteria from the NABIR bacterial transport field site at Oyster, VA

In addition to the above, representative subsets of isolates from UMTRA sites and the NABIR FRC site were being developed (by characterization of selected isolates) when funds for the project were reduced in August 2003.

8. Screening of SMCC Strains for Specific Characteristics

NABIR and other DOE-funded scientists expressed interest in isolates that reduce or accumulate metals and/or radionuclides, that are resistant to metals, or that degrade certain organic compounds. Because information on such traits was not present in the SMCC databases at the start of this project, the following groups of isolates were screened for various characteristics at the request of DOE-funded investigators during the period covered by this report.

- > 1,300 isolates from the Oyster Site in Virginia were screened for resistance to each of 8 antibiotics.
- > 500 isolates from deep aquifers at the Savannah River Site were screened for resistance to 8 antibiotics
- > 200 isolates from deep saturated lacustrine sediments, paleosols, and fluvial sands at the Hanford Site were screened for resistance to 8 antibiotics.
- > 1,000 isolates from the Oyster Site in Virginia were screened for the ability to degrade citrate under anaerobic conditions in the presence of nitrate.
- > 1,000 isolates from the Oyster Site in Virginia were screened for the ability to reduce iron and/or U(VI).
- > 60 isolates from UMTRA filed sites were screened for the ability to reduce iron or U(VI).

9. Phylogenetic Characterization of SMCC Strains

More than 1000 SMCC strains were characterized phylogenetically during the period covered by this report. Our detailed procedures for phylogenetic characterization are described in the original and renewal proposals for this project. Briefly, the complete or partial DNA base sequence for the 16S ribosomal RNA gene is determined for each strain. The resulting sequence is then analyzed with selected comparison sequences for known bacteria, using various types of algorithms that compute the phylogenetic (evolutionary) relatedness of the compared strains. This analysis provides information on the possible identity (or novelty) of the SMCC strains, and sometimes provides information on their likely physiological characteristics and capabilities. In general DOE-funded investigators have found the phylogenetic information to be more useful than physiological or morphological data when attempting to locate and select SMCC strains for use in their research programs. The specific groups of strains that were characterized in this way are summarized below. The 16S rRNA gene sequences have been made available to the scientific community at the GenBank and Ribosomal Database Project websites.

- 75 strains from deep aquifers at the Savannah River Site
- 175 strains from GEMHEX samples at the Hanford Site
- 69 strains from various locations at the Hanford Site (at the request of J. K. Fredrickson, PNNL)
- 257 strains from the Oyster site in Virginia (at the request of M. DeFlaun)
- 47 possible U(VI)- or Fe(III)-reducing strains from the Shiprock UMTRA site
- 130 strains from SX-108 tank soil at the Hanford Site (at the request of J. K. Fredrickson, PNNL)
- >100 possible Fe-reducing strains from the Oyster Site in Virginia

- >75 citrate-degrading strains from the Oyster Site Virginia (at the request of E. Murphy, PNNL)
- 42 strains from the NABIR FRC site at ORNL
- 85 antibiotic-resistant strains from the Oyster site in Virginia
- 15 strains of propane-oxidizing bacteria from the Dover Air Force Base site in Delaware (at the request of T. Palumbo, ORNL)

10. Development and Maintenance of a SMCC Website

A SMCC website was established for the use of NABIR Program investigators was established in 1999. This site included general information about the culture collection and its various databases, along with descriptions of the major sets of strains and the environments from which they were isolated. The site also contained a searchable database containing well-characterized strains from the Savannah River Site and the Hanford Site. Investigators could search rapidly for strains with certain characteristics or combinations of characteristics. Additional development of the website (which has since been removed from the web) to make it more useful to the NABIR Program was in progress when funds for the project were reduced in 2003.

11. Maintenance of SMCC Databases

Two major SMCC databases were maintained throughout the duration of the project: (1) the culture database, which contained all known characteristics of the strains themselves; and (2) the sample database, which contained information on the environments from which the cultures were isolated. Throughout the period covered by this report, these databases were updated as the appropriate information became available.

12. Archiving of SMCC Records

The SMCC had a rather large volume of on-paper chain-of-custody records documenting the origin, isolation history, and preservation procedures for each strain in the collection. All of these on-paper records were archived by electronic scanning and subsequent storage of multiple copies on compact discs (CDs) during the period covered by this report.

13. Quality Assurance/Quality Control (QA/QC Activities)

QA/QC measures included testing randomly selected SMCC strains for viability and purity, as detailed in the original and renewal proposals for this project. SMCC databases were also checked periodically for accuracy throughout the period covered by this report.

C. Supportive and Collaborative Research Activities

Throughout the duration of the project, funding was provided not only for maintenance and operation of the SMCC, but also for the PI (D. L. Balkwill) to carry out research in support of the NABIR program. Some of this research was carried out independently, and some of it was carried out in collaboration with other DOE-funded investigators. The principal supportive and collaborative efforts carried out during the period covered by this report are summarized below.

Additional information may be found in publications stemming from these research activities (see Products Delivered, below).

- **Research related to UMTRA field sites:** Aerobic, chemoheterotrophic bacteria in more than 50 water and sediment samples from the Shiprock and Gunnison UMTRA field research sites were enumerated by plating on appropriate media. Analyses of colony morphologies were carried out to determine the number of apparently distinct types of bacteria in each sample. Approximately 2,350 strains of bacteria were isolated form the plates used for enumeration and incorporated into the SMCC. Enrichments for Fe(III)- and U(VI)-reducing bacteria were carried out on selected samples from the Shiprock site. 47 possible metal-reducing bacteria were isolated from these enrichments. These isolates were characterized by determination and analysis of 16S rRNA gene sequences. Summary tables showing bacterial numbers and diversity in all of the UMTRA samples were provided to P. Long (PNNL), who coordinated the field research efforts at these sites.
- **Research related to the NABIR field site in Oyster, Virginia:** Aerobic, chemoheterotrophic bacteria in more than 70 water and sediment samples from the NABIR bacterial transport field site in Oyster, Virginia were enumerated by plating on appropriate media. (The total number of samples includes those taken for initial characterization of the site, those taken during transport experiments, and those obtained during the final monitoring phase of the project.) Analyses of the colony morphologies were also carried, to determine the number of apparently distinct types of bacteria in each sample. Summary tables showing the numbers and diversity of aerobic bacteria in the Oyster samples were provided to all PIs involved with research at the site. Approximately 1,700 bacterial strains were isolated from the Oyster samples and incorporated into the SMCC. As noted earlier, many Oyster isolates were screened for the ability to reduce Fe(III) or U(VI), the ability to degrade citrate, and resistance to antibiotics, in order to meet the needs of the research plan at the Oyster site. In addition, enrichments for were Fe(III)- and U(VI)-reducing bacteria were carried out and produced 65 possible metal-reducing strains. These and many other Oyster isolates were characterized phylogenetically by analysis of 16S rRNA gene sequences.
- **Research on bacteria in Columbia River sediments at the Hanford Site (collaboration with J. Fredrickson, J. Zachara, and others at PNNL):** T-RFLP (terminal restriction fragment length polymorphism) and other direct molecular biological analyses were carried out on one water and nine sediment samples from the hyphoreic zone of the Columbia River at the Hanford Site. The purpose of this research was to characterize the microbial communities in these previously unstudied materials and to evaluate different methods for collection and preservation of samples.
- **Research on bacteria in soils contaminated with radionuclides at the Hanford Site (collaboration with J. Fredrickson and others at PNNL):** As noted above, we characterized 130 bacterial strains isolated from SX-108 tank soil at the Hanford Site by phylogenetic analysis of 16S rRNA gene sequences. The purpose of this research was to characterize the microbial communities in the contaminated soils and to determine whether previously unreported radiation-resistant strains were present.

- **Research on bacteria in sediment samples and enrichment cultures of sediments from the NABIR FRC site at ORNL (collaboration with J. Kostka, Florida State Univ.):** As noted above, 42 possible Fe(III)-reducing isolates from samples or enrichment cultures from the FRC were characterized by analysis of their 16S rRNA gene sequences. In addition, direct analyses of microbial communities were carried out on sediment samples and enrichments by extraction of community DNA, followed by polymerase chain amplification, cloning, and sequencing of 16S rRNA genes. These studies served to identify the types of bacteria that were present under differing conditions in sediments and enrichments cultures, and to provide information on possible strategies for biostimulation of uranium reduction in FRC sediments.

Additional, less extensive collaborations were carried out with T. Barkay (Rutgers Univ. – metal-resistant bacteria from the Savannah River Site), D. Boone (Oregon Graduate Univ. – novel species of anaerobic bacteria), D. Chandler (PNNL – comparison of molecular methods for characterization of microbial communities in environmental samples), M. Gershwin (Univ. of California-Davis – possible connection between aromatic-degrading subsurface bacteria and biliary cirrhosis), R. Miller (Oklahoma State Univ. – phylogenetic characterization of subsurface bacteria by analysis of gyrase B sequences), R. Tanner (Univ. of Oklahoma – novel anaerobic bacteria), and V. Vairavamurthy (Brookhaven National Laboratory – characterization of a new *Klebsiella* strain).

D. Reduction and Closure of the SMCC

After DOE decided not to continue funding for the SMCC, the collection was inventoried to identify strains that could be eliminated because they are likely to be duplicates or near-duplicates of other strains in the collection, or because they did not come from overly deep or interesting subsurface environments. The main purpose for elimination of unneeded strains was to minimize the cost of maintaining the collection without DOE support, if the Florida State University decided to do so. Approximately 7,000 strains were eliminated from the collection's holdings (*i.e.*, removed from stocks held at -80C and stocks stored under liquid nitrogen), with approval of the DOE program manager for the project. Approximately 8,800 strains remained in the collection after this exercise was completed. The remaining strains and the records associated with them were then transported to the PI's new laboratory in the College of Medicine research building at Florida State.

For the time being, Florida State University has decided to maintain the remaining SMCC cultures (*i.e.*, continue to provide protected freezer space and liquid nitrogen storage). However, the collection will not function as an operational facility that provides cultures and information to the scientific community in the absence of extramural funding. The remaining cultures simply will be stored until the university decides their ultimate fate. We currently plan to seek funding for support of a functional collection from the National Science Foundation or other sources.

E. Products Delivered

1. Peer-Reviewed Journal Articles

The following peer-reviewed journal articles describing research that was supported (entirely or in part) by SMCC funding appeared or were accepted for publication during the period covered by this report.

1. Balkwill, D.L., G.R. Drake, R.H. Reeves, J.K. Fredrickson, D.C. White, D.B. Ringelberg, D. P. Chandler, M.F. Romine, D.W. Kennedy, and C.M. Spadoni. 1997. Taxonomic study of aromatic-degrading bacteria from deep terrestrial subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov. *Int. J. Syst. Bacteriol.* 47:191-201.
2. Balkwill, D. L., R. H. Reeves, G. R. Drake, J. Y. Reeves, F. H. Crocker, M. B. King, and D. R. Boone. 1997. Phylogenetic characterization of bacteria in the Subsurface Microbial Culture Collection. *FEMS Microbiol. Rev.* 20:201-216.
3. Colwell, F.S., T.C. Onstott, M.E. Delwiche, D. Chandler, J.K. Fredrickson, Q.-J. Yao, J.P. McKinley, D.R. Boone, R. Griffiths, T.J. Phelps, D. Ringelberg, D.C. White, L. LaFreniere, D. Balkwill, R.M. Lehman, J. Konisky, and P.E. Long. 1997. Microorganisms from deep, high-temperature sandstones: constraints on microbial colonization. *FEMS Microbiol. Rev.* 20:425-435.
4. Liu, Y., T.M. Karnauchow, K.E. Jarrell, D.L. Balkwill, G.R. Drake, D. Ringelberg, R. Clarno, and D.R. Boone. 1997. Description of two new thermophilic species of *Desulfotomaculum, Desulfotomaculum putei* sp. nov. from the deep terrestrial subsurface and *Desulfotomaculum luciae* sp. nov. from a hot spring. *Int. J. Syst. Bacteriol.* 47:615-621.
5. Franzmann, P. D., Y. Liu, D. L .Balkwill, H. C. Aldrich, E. Conway de Macario, and D. R. Boone. 1997. *Methanobacterium frigidum* sp. nov., a psychrophilic, H_2-using methanogen from Ace Lake, Antarctica. *Int. J. Syst. Bacteriol.* 47:1068-1072.
6. Chandler, D. P., S.-M. Li, C. M. Spadoni, G. R. Drake, D. L. Balkwill, J. K. Fredrickson, and F. J. Brockman. 1997. A molecular comparison of culturable aerobic heterotrophic bacteria and 16S rDNA clones derived from a deep subsurface sediment. FEMS Microbiol. Ecol. 23:131-144.
7. Balkwill, D.L., E.M. Murphy, D.M. Fair, D.B. Ringelberg, and D.C. White. 1998. Microbial communities in high and low recharge environments: implications for microbial transport in the vadose zone. *Microb. Ecol.* 35:156-171.
8. Onstott, T.C., T.J. Phelps, F.S. Colwell, D. Ringelberg, D.C. White, D.R. Boone, J.P. McKinley, T.O. Stevens, P.E. Long, D.L. Balkwill, W.T. Griffin, and T. Kieft. 1998. Observations pertaining to the origin and ecology of microorganisms recovered from the deep subsurface of Taylorsville Basin, Virginia. *Geomicrobiol. J.* 15:353-385.
9. Liu, Y., D.L. Balkwill, H.C. Aldrich, G.R. Drake, and D.R. Boone. 1999. Characterization of the aneraerobic propionate-degrading syntrophs *Smithella propionica* gen. nov. sp. nov. and *Syntrophobacter wolinii*. *Int. J. Syst. Bacteriol.* 49:545-556.

10. Fredrickson, J.K., D.L. Balkwill, M.F. Romine, and T. Shi. 1999. Ecology, physiology, and phylogeny of deep subsurface *Sphingomonas* sp. *J. Ind. Microbiol. Biotechnol.* 23:273-283.

11. Crocker, F.H., J.K. Fredrickson, D.C. White, D.B. Ringelberg, and D.L. Balkwill. 2000. Phylogenetic and physiological diversity of *Arthrobacter* strains isolated from unconsolidated subsurface sediments. *Microbiol.* 145:1295-1310.

12. Van Waasbergen, L.G., D.L. Balkwill, F.S. Crocker, B.N. Bjornstad, and R.V. Miller. 2000. Genetic diversity among *Arthrobacter* species collected across a heterogeneous series of terrestrial deep-subsurface sediments as determined on the basis of 16S rRNA and *recA* gene sequence. *Appl. Environ. Microbiol.* 66:3454-3463.

13. Sharma, P.K., D.L. Balkwill, A. Frenkel, and V.A. Vairavamurthy. 2000. A new *Klebsiella planticola* strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. *Appl. Environ. Microbiol.* 66:3083-3087.

14. Fuller, M.E., S.H. Streger, R.K. Rothmel, B.J. Mailloux, J.A. Hall, T.C. Onstott, J.K. Fredrickson, D.L. Balkwill, and M.A. DeFlaun. 2000. Development of a vital fluorescent staining method for monitoring bacterial transport in subsurface environments. *Appl. Environ. Microbiol.* 66:4486-4496.

15. Shi, T., J.K. Fredrickson, and D.L. Balkwill. 2001. Biodegradation of polycyclic aromatic hydrocarbons by *Sphingomonas* strains isolated from the terrestrial subsurface. *J. Ind. Microbiol. Biotechnol.* 26:283-289.

16. Rogel, A.M., I. Hernandez-Lucas, L.D. Kuykendall, D.L. Balkwill, and E. Martinez-Romero 2001. Nitrogen-fixing nodules with *Ensifer adhaerens* harboring *Rhizobium tropici* symbiotic plasmids. *Appl. Environ. Microbiol.* 67:3264-3268.

17. DeFlaun, M.F., M.E. Fuller, P. Zhang, W.P. Johnson, B.J. Mailloux, W.E. Holben, W.P. Kovacik, D.L. Balkwill, and T.C. Onstott. 2001. Comparison of methods for monitoring bacterial transport in the subsurface. *J. Microbiol. Meth.* 47:219-231.

18. Balkwill, D., J. Chen, M. DeFlaun, F. Dobbs, H. Dong, J. Fredrickson, M. Fuller, M. Green, T. Ginn, T. Griffin, W. Hoben, S. Hubbard, W. Johonson, P. Long, B. Mallioux, E. Majer, M. McInerny, C. Murray, T. Onstott, T. Phelps, T. Scheibe, D. Swift, D. White, and F. Wobber. 2001. Breakthroughs in field-scale bacterial transport. *Eos* 82:417, 423-425.

19. Benyehuda, G., J. Coombs, P.L. Ward, D.L. Balkwill, and T. Barkay. 2003. Metal resistance among aerobic chemoheterotrophic bacteria from the deep terrestrial subsurface. *Can. J. Microbiol.* 49:151-156.

20. Petrie, L., N.N. North, S.L. Dollhopf, D.L. Balkwill, and J.E. Kostka. 2003. Enumeration and characterization of iron(III)-reducing microbial communities from acidic subsurface sediments contaminated with uranium(VI). *Appl. Environ. Microbiol.* 69:7467-7479.

21. Moser, D., Onstott, T.C., J.K. Fredrickson, F.J. Brockman, D.L. Balkwill, G.R. Drake, S.M. Pfiffner, and D.C. White. 2003. Temporal shifts in the geochemistry and microbial community structure of an ultradeep mine borehole following isolation. *Geomicrobiol. J.* 20:517-548.

22. Selmi, C., D.L. Balkwill, P. Invernizzi, A.A. Ansari, R.L. Coppel, M. Podda, P.S. Leung, T.P. Kenny, J. Van de Water, M.H. Nantz, M.J. Kurth, and M.E. Gershwin. 2003. Patients

with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. *Hepatology* 38:1250-1257.

23. Balkwill, D.L., T.L. Kieft, T. Tsukuda, H.M. Kostanderithes, T.C. Onstott, S. Macnaughton, J. Bowans, and J.K. Fredrickson. 2004. Identification of iron-reducing *Thermus* strains as *Thermus scotoductus*. *Extremophiles* 8:37-44.

24. Fredrickson, J.K., J.M. Zachara, D.L. Balkwill, D. Kennedy, S.W. Li, H.M. Kostandarithes, M.J. Daly, M.F. Romine, and F.J. Brockman. 2004. Geomicrobiology of high-level nuclear waste contaminated vadose sediments at the Hanford Site, Washington State. *Appl. Environ. Microbiol.* 70:4230-4241.

25. North, N.N., S.L. Dollhopf, L. Petrie, J.D. Istok, D.L. Balkwill, and J.E. Kostka. 2004. Change in bacterial community structure during in situ biostimulation of subsurface sediment cocontaminated with uranium and nitrate. *Appl. Environ. Microbiol.* 70:4911-4920.

26. Padgett, K.A., C. Selmi, T.P. Kenny, P.S. Leung, D.L. Balkwill, A.A. Ansari, R.L. Coppel, and M.E. Gershwin. 2005. Phylogenetic and immunological definition of four lipoylated proteins from *Novosphigobium aromaticivorans*, implications for primary biliary cirrhosis. *J. Autoimmun.* 24:209-219.

27. Liou, J.S., D.L. Balkwill, G.R. Drake, and R.S. Tanner. 2005. *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. *Int. J. Syst. Evol. Microbiol.* 55:2085-2091.

2. Peer-Reviewed Book Chapters

The following peer-reviewed book chapters describing research that was supported (entirely or in part) by SMCC funding appeared or were accepted for publication during the period covered by this report.

1. Balkwill, D.L., and D.R. Boone. 1997. Identity and diversity of microorganisms cultured from subsurface environments. *In:* P.S. Amy and D. L. Haldeman (eds.), *The Microbiology of the Terrestrial Deep Subsurface*, pp. 105-117. CRC Lewis Publishers, Boca Raton, Florida.

2. Balkwill, D.L. 2005. Genus VI – *Ensifer*. *In:* D.J. Brenner, N.R. Krieg, and J.T. Staley (eds.), *Bergey's Manual of Systematic Bacteriology, 2nd Ed., Vol. 2, Part C: The Alpha-, Beta-, Delta- and Epsilonproteobacteria*, pp. 354-358. Springer, New York.

3. Balkwill, D.L. 2002. Subsurface microbial communities: diversity of culturable microorganisms. *In:* G. Bitton (ed.) *Encyclopedia of Environmental Microbiology*, pp. 3065-3071, John Wiley & Sons, New York.

4. Balkwill, D.L., J.K. Fredrickson, and M.F. Romine. 2003. *Sphingomonas* and related genera: *In:* M. Dworkin (ed.), *The Prokaryotes*, Springer-Verlag, New York.

5. Balkwill, D.L. 2004. Spatial segregation: the deep subsurface story. In: R.V. Miller and M.J. Day (eds.), *Microbial Evolution: Gene Establishment, Survival and Exchange*, pp. 214-230. ASM Press, Washington, D.C.

3. Published Abstracts

The following published abstracts describing research that was supported (entirely or in part) by SMCC funding and that was presented at national or international meeting appeared in print during the period covered by this report.

1. Balkwill, D.L. 1996. Phylogenetic characterization of microorganisms isolated from subsurface environments. *Abstr. 1996 Int. Symp. Subsurface Microbiol.*, Davos, Switzerland.
2. Colwell, F., J.K. Fredrickson, D. Chandler, M. Delwiche, T.C. Onstott, Q.-J. Yao, T. Phelps, R. Griffiths, D. Ringelberg, D. White, J. Konisky, D. Boone, D. Balkwill, and R. Lehman. 1996. Microorganisms from deep, high-temperature, Cretaceous sandstones. *Abstr. 1996 Int. Symp. Subsurface Microbiol.*, Davos, Switzerland.
3. Drake, G.R., D.L. Balkwill, D.R. Boone, and T.J. Phelps. 1996. Phylogenetic characterization of anaerobic microorganisms from the deep subsurface. *Abstr. 1996 Int. Symp. Subsurface Microbiol.*, Davos, Switzerland.
4. Fair, D.M., I.R. Guttmann, V.L. Arunakul, and D.L. Balkwill. 1996. Phylogenetic characterization of subsurface bacteria by analysis of 16S ribosomal RNA and gyrase B gene sequences and phenotypic profiles. *Abstr. 1996 Int. Symp. Subsurface Microbiol.*, Davos, Switzerland.
5. Liu, Y., D.L. Balkwill, H.C. Aldrich, and D.R. Boone. 1998. A novel anerobic syntrophic bacterium that degrades propionate and produces butyrate. *Abstr. 98th Gen. Meeting Amer. Soc. for Microbiol.*, Atlanta, Georgia.
6. Van Waasbergen, L.G., D.L. Balkwill, and R.V. Miller. 1998. Analysis of vertical phylogenetic patterns in terrestrial deep-subsurface *Arthrobacter* populations. *Abstr. 98th Gen. Meeting Amer. Soc. for Microbiol.,* Atlanta, Georgia.
7. DeFlaun, M.F., S. Kotelnikova, D. Balkwill, S. Streger, T.C. Onstott, and D. Moser. 1999. Metabolic diversity in ultra-deep groundwater. *Abstr. 1999 Int. Symp. Subsurface Microbiol.,* Vail, CO.
8. Bueker, C.L., G.R. Drake, M.F. DeFlaun, S. Streger, and D.L. Balkwill. 1999. Phylogenetic characterization of bacterial in Atlantic Coastal Plain sediments at a site in Virginia. *Abstr. 1999 Int. Symp. Subsurface Microbiol.*, Vail, CO.
9. Drake, G.R., M.F. DeFlaun, S. Streger, S.B. Levy, C.L. Bueker, and D.L. Balkwill. 1999. Widespread antibiotic resistance among bacteria isolated from subsurface environments. *Abstr. 1999 Int. Symp. Subsurface Microbiol.*, Vail, CO.
10. Li, S.W., C.M. Spadoni, J.K. Fredrickson, D.R. Geist, and D.L. Balkwill. 2001. Microbial and geochemical characteristics across the goundwater – Columbia River water boundary. *Abstr. 101st Gen. Meeting Amer. Soc. for Microbiol.*, Orlando, FL.
11. Kennedy, D.W., S.W. Li, C.W. Lindenmeier, D.L. Balkwill, and J.K. Fredrickson. 2001. Microbiological properties of radioactive vadose sediments from the Hanford Site. *Abstr. 101st Gen. Meeting Amer. Soc. for Microbiol.*, Orlando, FL.

12. Dalton, D.D., J.E. Kostka, G.R. Drake, K. Scheinemann, and D.L. Balkwill. 2001. Gram-positive dissimilatory Fe(III)-reducing bacteria isolated from agricultural soils and subsurface sediments. *Abstr. 101st Gen. Meeting Amer. Soc. for Microbiol.*, Orlando, FL.

13. Drake, G.R., D. Dalton, K. Scheinemann, J.E. Kostka, and D.L. Balkwill. 2001. Diversity of dissimilatory Fe(III)-reducing bacteria from soils and subsurface sediments. *Abstr. 8th Int. Symp. Microb. Ecol.*, Amsterdam.

13. Kostandarithes, H., A. Eakin, D. Balkwill, M. Daly, D. Kennedy, S. Li, and J. Fredrickson. 2002. Characterization of bacteria isolated from radionuclide-contaminated subsurface vadose sediments. *Abstr. 102nd Gen. Meeting Amer. Soc. for Microbiol.*, Salt Lake City, UT.

14. Kostka, J., L. Petrie, S. Dollhopf, N. North, and D. Balkwill. 2002. Enumeration and characterization of Fe(III)- and U(VI)-reducing bacteria in subsurface sediments from the U.S. DOE-NABIR Field Research Center (FRC) at Oak Ridge, Tennessee. *Abstr. 102nd Gen. Meeting Amer. Soc. for Microbiol.*, Salt Lake City, UT.

15. J. Shutthanandan, X. Yin, G. Drake, D. Oliver, T. Kieft, D. Balkwill, and F. Brockman. 2003. Differential community structure in vadose zone microcosms supporting and nor supporting chromium reduction during bioremediation. *Abstr. 103rd Gen. Meeting Amer. Soc. for Microbiol.*, Washington, D.C.

16. G. Zhang, H. Dong, Z. Xu, J. Yang, Z. Zhang, C. Cohen, and D. Balkwill. 2003. Microbial community in ultra-high pressure metamorphic rocks from Chinese continental scientific deep drilling. *Abstr. 103rd Gen. Meeting Amer. Soc. for Microbiol.* Washington, D.C.

17. Kostka, J., N. North, E. Petrie, S. Dollhopf, and D. Balkwill. 2003. Characterization of iron(III)-reducing microbial communities from acidic subsurface sediments contaminated with uranium at the U.S. DOE-NABIR Field Research Center (CRC). *Abstr. 103rd Gen. Meeting. Amer. Soc. for Microbiol.*, Washington, D.C.

18. Kostka, J., N. North, S. Dollhopf, L. Petrie, and D. Balkwill. 2004. In situ change in metal-reducing bacteria and other members of the sedimentary microbial community during biostimulation of the acidic subsurface. *Abstr. 104th Gen. Meeting Amer. Soc. for Microbiol.*, New Orleans, LA.