DOE/ER/62534-1

VADOSE ZONE MICROBIAL COMMUNITY STRUCTURE AND ACTIVITY IN METAL/RADIONUCLIDE CONTAMINATED SEDIMENTS

DOE Grant No. DE-FG02-98ER62534
Florida State University

Final Technical Report

INTRODUCTION

This was a collaborative research project involving Florida State University (Dr. David Balkwill, PI), New Mexico Institute of Mining and Technology (Dr. Tom Kieft, PI), and Pacific Northwest National Laboratory (Dr. Fred Brockman, PI). The three institutions worked together throughout the duration of project, with Dr. Brockman functioning as lead PI and overall project manager, but they were funded separately under distinct DOE grants numbers.

The project was initially funded for three years, from 11/15/97 through 11/14/00. A no-cost extension was then granted, from 11/15/00 through 11/14/01. An interim technical report describing much of the research performed under the no-cost extension was submitted via the Research Information Management System (RIMS) on 9/13/01 (copy attached as Appendix I). This final technical report describes the additional research carried out at Florida State University since the last RIMS interim report was submitted.

PROJECT GOALS

The primary goals of this project were to:

(1) Determine the potential for transformation of Cr(VI) (oxidized, mobile) to Cr(III) (reduced, immobile) under unsaturated conditions as a function of different levels and combinations of (a) chromium, (b) nitrate (co-disposed with Cr), and (c) molasses (inexpensive bioremediation substrate), and...

(2) Determine population structure and activity in experimental treatments by characterization of the microbial community by signature biomarker analysis and by RT-PCR and terminal restriction fragment length polymorphism (T-RFLP) of 16S ribosomal RNA genes.

PROGRESS

It was determined early in the one-year no-cost extension period of the project that the T-RFLP approach was problematic in regard to providing information on the identities of microorganisms in the samples examined (see report in Appendix I). As a result, it could not provide the detailed information on microbial community structure that was needed to assess the effects of treatments with chromium, nitrate, and/or molasses. Therefore, we decided to obtain the desired information by amplifying (using TR-PCR, with the same primers used for T-RFLP) and cloning 16S rRNA gene sequences from the same RNA extracts that were used for T-RFLP analysis. We

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also decided to use a restriction enzyme digest procedure (fingerprinting procedure) to place the clones into types, so representatives of each type could be sequenced. (This avoids the unnecessary sequencing of substantial numbers of duplicate clones.)

The primary focus of the research carried out at Florida State University during the period covered by this report was twofold: (a) to complete the sequencing of the clones (which had been started shortly before the last interim RIMS report, in Appendix I, was submitted), and (b) to analyze the clone sequences phylogenetically in order to determine the relatedness of the bacteria detected in the samples to each other and to previously described genera and species.

Screening and Sequencing of Clones

A total of 575 randomly selected clones were analyzed by restriction enzyme digestion. These were found to include 56 distinct types. 112 clones – two representative examples from each of the clone types detected by restriction analysis – were selected for sequencing at Florida State University. Approximately 1,000 bases of sequence (roughly two-thirds of the 16S rRNA gene) were generated for each of the selected clones during the period covered by this report. It was not possible to generate a clean, analyzable sequence from a small number of the clones, owing to various technical difficulties. In such cases, alternate clones from the same restriction digest types were selected to replace the clones that could not be sequenced.

The 100+ clone sequences were edited and evaluated in the context of the secondary structure of the 16S ribosomal RNA molecule, in order to ensure proper base pairing in paired regions and to facilitate later alignment of sequences for more detailed phylogenetic analyses. The evaluated and edited sequences were then subjected to a Similarity Rank analysis, using the web site of the Ribosomal Database Project, in order to obtain preliminary information on the likely identities of the clones. This analysis indicated that most of the clones belonged to three major subdivisions of the bacteria, as follows:

37%	Alpha Subdivision of the *Proteobacteria*
38%	Beta Subdivision of the *Proteobacteria*
23%	High-G+C Subdivision of the Gram-positive bacteria
2%	Other

Phylogenetic Analysis of Clone Sequences

The clone sequence were aligned to comparison sequences from public databases (chosen based on the results of the above Similarity Rank analysis) and analyzed phylogenetically, to determine how the clones were related to each other and to previously described bacteria in an evolutionary sense. Analyses were carried out with distance matrix, parsimony, and maximum likelihood methods, all of which produced diagrams called phylogenetic trees, in which the evolutionary relationships among the organisms examined are illustrated by the branching patterns, clustering of organisms together, and the lengths of the horizontal branch to which the individual organisms are assigned. Sample phylogenetic trees, produced by the distance matrix method are provided in Appendix II.

Clones that were assigned to the alpha subdivision of the *Proteobacteria* were most closely related to species of the following genera: *Aminobacter, Brevundimonas, Devosia, Mesorhizaobium,* and *Phenylobacterium.* As is frequently the case with clone sequences amplified directly from environmental samples, some groups of clones were phylogenetically quite distinct from the closest named genera, indicating that they may represent novel genera within the alpha subdivision of the *Proteobacteria.* Other clones, however, were virtually indistinguishable from the most closely related comparison organisms (*e.g.*, those most closely related to *Aminobacter* and *Mesorhizobium*).

Clones that were assigned to the beta subdivision of the *Proteobacteria* were most closely related to species of the following genera: *Acidovorax, Aquabacterium, Herbaspirillum, Leptothrix, Ralstonia, Rubrivivax,* and *Variovorax.* As with the alpha-*Proteobacteria* clones, some of the beta-*Proteobacteria* clones were phylogenetically quite distinct from the closest named genera, indicating that they may represent novel genera within the beta subdivision of the *Proteobacteria.*

Clones that were assigned to the high-G+C subdivision of the Gram-positive bacteria were not overly diverse. All of these clones were most closely related either to *Amycolatopsis* or *Cellulomonas.* In this case, the clones were phylogenetically very close to certain species within these two genera and did not appear to be novel species or genera.

A small number of clones were assigned to the Planctomyces and Relatives subdivision of the bacteria. These clones were quite distant from all known organisms within this group and are very likely to represent previously unreported genera or even higher-level taxa.

The above analysis also indicated that there were some clear differences in the composition of the microbial communities in sediments that received different treatments (*i.e.*, chromium, nitrate, and/or molasses; see above). Some phylogenies were detected only in one treatment, while others were detected only in two treatments. Very few were detected in more than two different treatments. In general, these results confirm the preliminary conclusions presented in the most recent RIMS interim report; *i.e.*, they indicate that addition of molasses and nitrate to the vadose zone has good potential to decrease the unsaturated transport of chromium into underlying aquifers, by stimulating the growth and metabolic activities of bacteria that reduce Cr(VI) to Cr(III).

Deliverables

Collaborative manuscripts describing the final results and conclusions of this project are now in preparation.

Appendix I

Copy of Most Recent Interim Technical Report for this Project

Submitted via Research Information Management System (RIMS) on 9/13/01

RIMS
RESEARCH INFORMATION MANAGEMENT SYSTEM

Projects

Action Due Now
All My Projects

Project Detail

Proposal
Award
Progress
Lead PI Info
Msg to Prgm Mgr

Other Items

FAQs
My Preferences
Change Password
Logout

Help

Award Information - ER62534-1010350-0002131

ID: ER62534-1010350-0002131
Principal Investigator: David L. Balkwill (850) 644-5719
Co-PIs:
Institution: Florida State University
Title: Vadose Zone Microbial Community Structure and Activity in Metal/Radionuclide-Contaminated Sediments
SC Division: SC-74
Program Manager: Anna C. Palmisano 301-903-9963
Research Areas: NABIR

Project Progress

Most recent report of results to date:

This is a joint project with Fred Brockman, Pacific Northwest National Laboratory and Tom Kieft, New Mexico Tech.

The goals of this project were to:
1) Determine the potential for transformation of Cr(VI) (oxidized, mobile) to Cr(III) (reduced, immobile) under unsaturated conditions as a function of different levels and combinations of (a) chromium, (b) nitrate (co-disposed w/ Cr), and (c) molasses (inexpensive bioremediation substrate), and
2) Determine population structure and activity in experimental treatments by characterization of the community by signature biomarker analysis and by RT-PCR and terminal restriction fragment length polymorphism (TRFLP) of rRNA.

Progress
Progress in the last 12 months, under a no cost extension, has focused on providing a phylogenetic context to previously completed joint microbiology-geochemical-hydrologic data sets from two multi-column studies. While TRFLP data on the microbial communities was collected from the columns, this data does not provide rigorous identification of the microbes. At best, TRFLP data alone provides a putative identification of a microbe based on searching sequence databases for other microorganisms with a terminal 16S rDNA fragment (TRF) of the same size. This approach has several problems including identifying a TRF from the sample with multiple (sometimes phylogenetically distant) genera with the same-sized TRF, and the assumption that the TRF from the sample represents a previously sequenced microorganism. To provide a rigorous phylogenetic context to the studies we have been amplifying (by RT-PCR, with the same primers) and cloning 16S sequences from the same RNA extracts that were used for TRFLP analysis, using restriction enzyme digestion to place the clones into types, and sequencing several representatives from each type. The sequences will enable us to cross-reference sequences to TRFs and to phylogenetically identify the organism associated with a TRF.

In the first multi-column study (6 columns fed chromium and different combinations and levels of molasses and nitrate), a total of 575 randomly selected clones were analyzed by restriction digests and found to contain 56 types. Sequencing of approximately 200 of the sequences is in progress. Clone libraries have been

constructed for the second six-column study (a comparison of no flow, unsaturated flow, and saturated flow in the absence of added nutrients) and restriction digest screening is being performed.

A second area of focus has been completion of the TRFLP analysis. The GelCompare software was purchased, expertise in running the software was developed, TRFLP data for 115 samples was re-analyzed, and similarity dendrograms constructed.

Most recent products delivered:

Presentations
1. Vadose zone chromium reduction in unsaturated batch and unsaturated flow column experiments. FJ Brockman, DL Balkwill, and TL Kieft. NABIR PI mtg, March 12-14, 2001 (Warrenton, VA)
2. Comparison of the microbial community under no flow, unsaturated flow, and saturated flow in columns. X Yin, DS Oliver, TL Kieft, and FJ Brockman. . American Society for Microbiology annual meeting, May 20-24 2001 (Orlando, FL).
3. Effects of unsaturated flow on hexavalent chromium reduction and 16s rRNA profiles. FJ Brockman, DS Oliver, X Yin, and TL Kieft. American Society for Microbiology annual meeting, May 20-24 2001 (Orlando, FL).
4. Microbial reduction of hexavalent chromium under vadose zone conditions. DS Oliver, FJ Brockman, RS Bowman, and TL Kieft. Soil Science Society of America annual meeting, October 2001.

Manuscripts
1. Oliver D, TL Kieft, DL Balkwill, and FJ Brockman. Microbial reduction of hexavalent chromium in sediments during unsaturated flow. Submitted, Environmental Science and Technology.
2. Yin X, D Oliver, TL Kieft and FJ Brockman. Effects of carbon and nitrate loading on hexavalent chromium reduction and 16S rRNA profiles in unsaturated batch and unsaturated flow columns. In preparation, Applied and Environmental Microbiology.
3. Brockman FJ, X Yin, D Oliver, and TL Kieft. Changes in 16S rRNA profiles in vadose zone sediment under no flow, unsaturated flow, and saturated flow conditions. In preparation, Microbial Ecology.

Most recent notes concerning the project:

Significance of Research
These results indicate that addition of molasses and nitrate to the vadose zone has good potential to decrease the unsaturated transport of chromium into underlying aquifers. To our knowledge, this work represents the first study of chromium reduction in subsurface sediments (versus surface soil) under unsaturated conditions.

Other Project Information Sources:

Project URL:

None

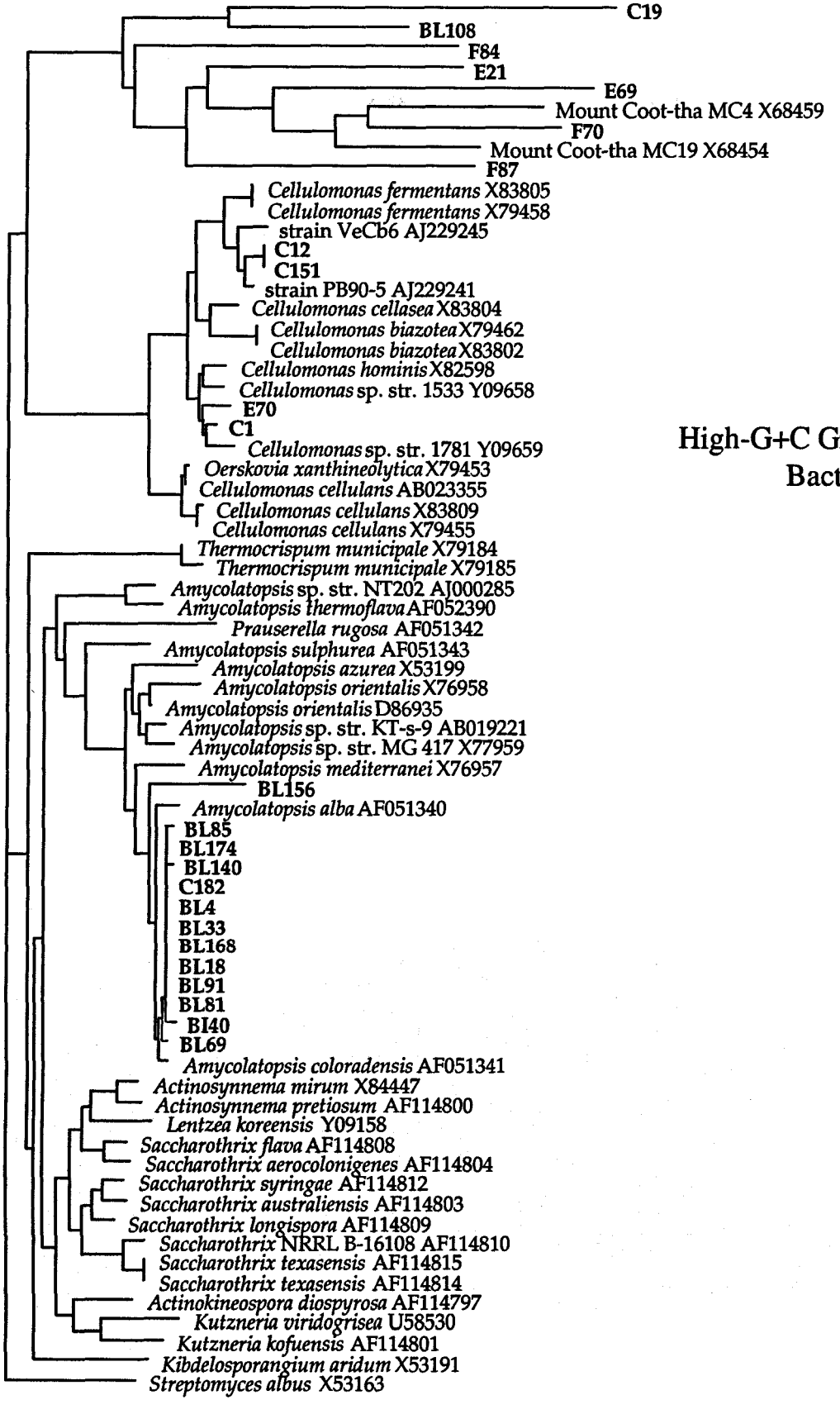
Related URL at institution:

http://www.fsu.edu/~biology/

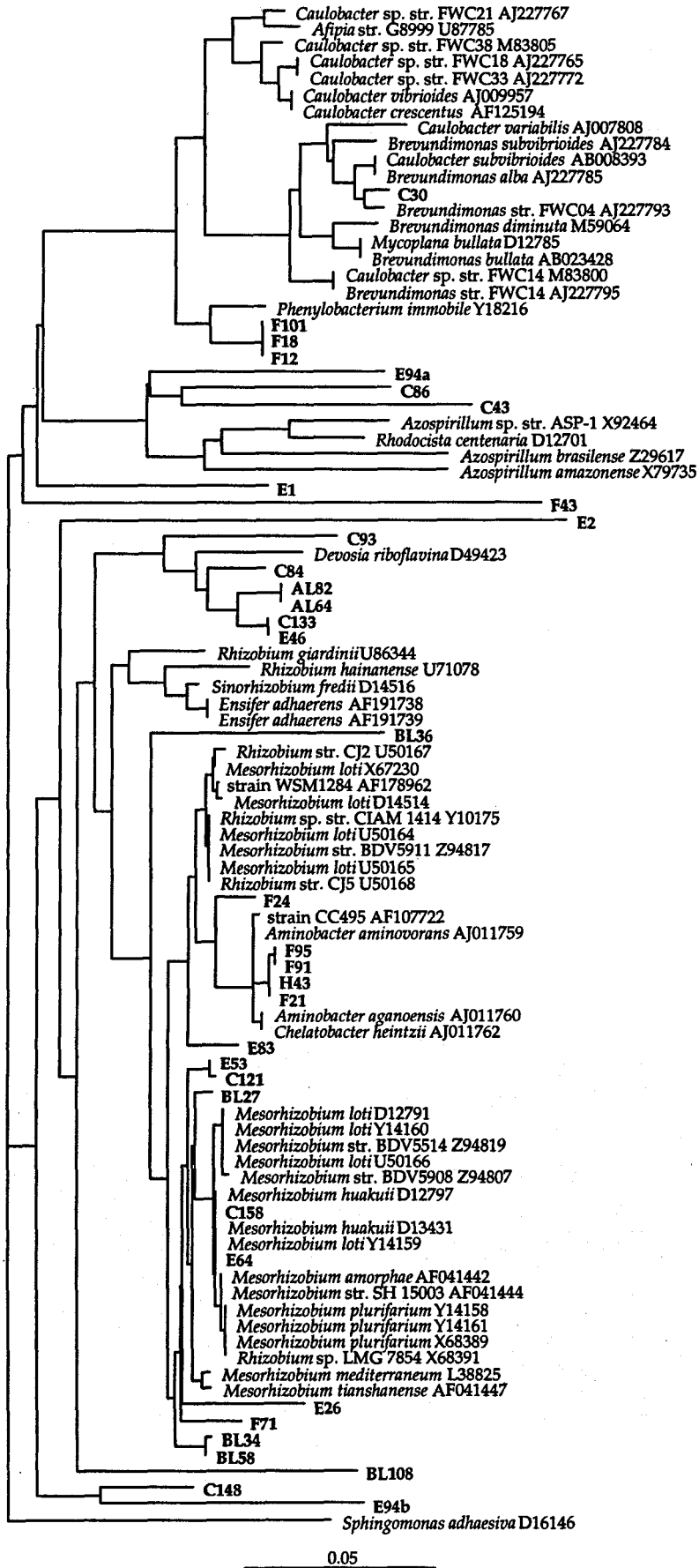
Appendix II

Examples of Phylogenetic Trees Produced by

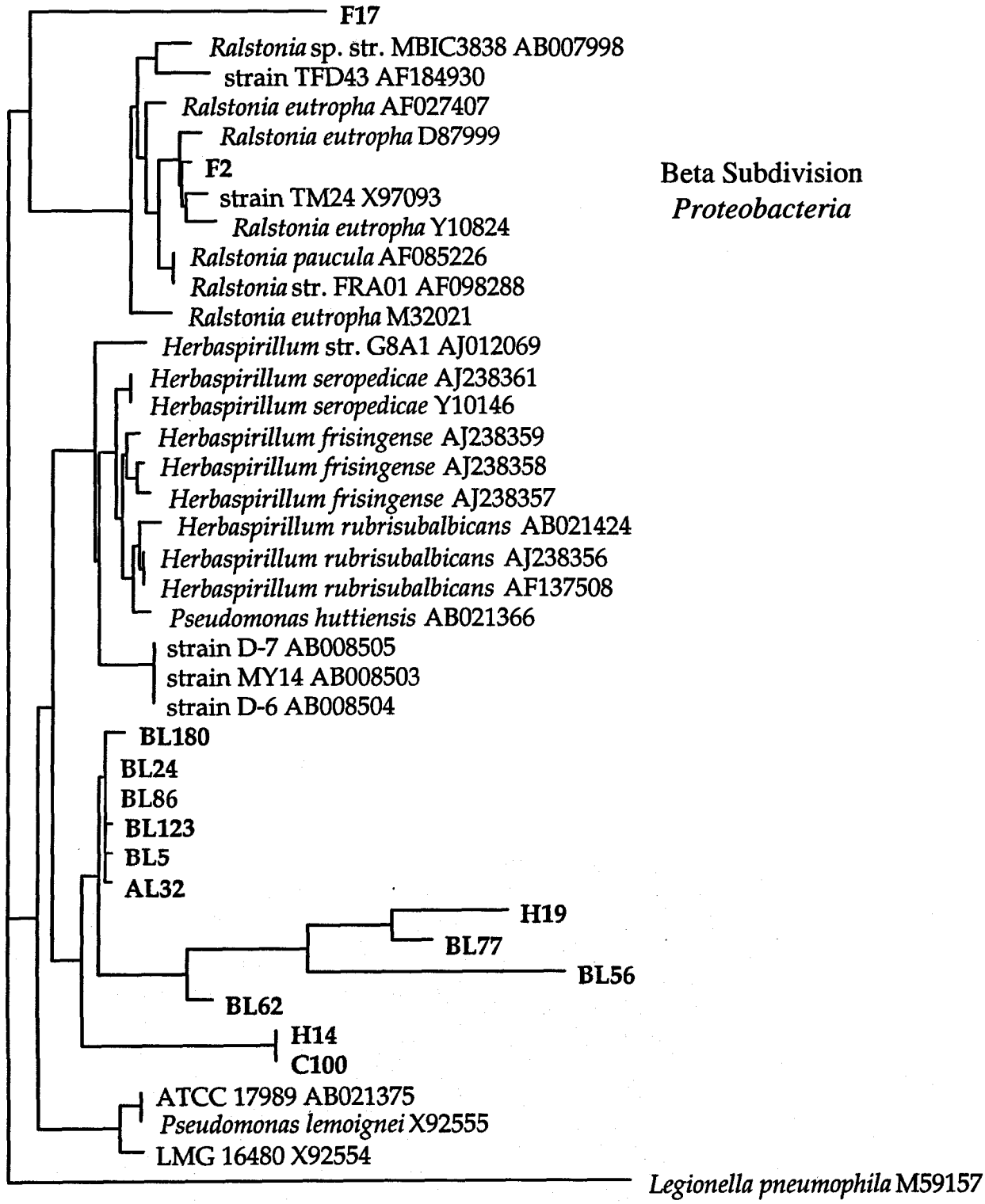
Distance-Matrix Analysis of 16S rRNA Clone Sequences



High-G+C Gram-Positive Bacteria



Alpha Subdivision
Proteobacteria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Ralstonia eutropha Y10824
Ralstonia paucula AF085226
Ralstonia str. FRA01 AF098288
Ralstonia eutropha M32021
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Herbaspirillum frisingense AJ238359
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strain D-7 AB008505
strain MY14 AB008503
strain D-6 AB008504
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BL86
BL123
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AL32
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Proteobacteria
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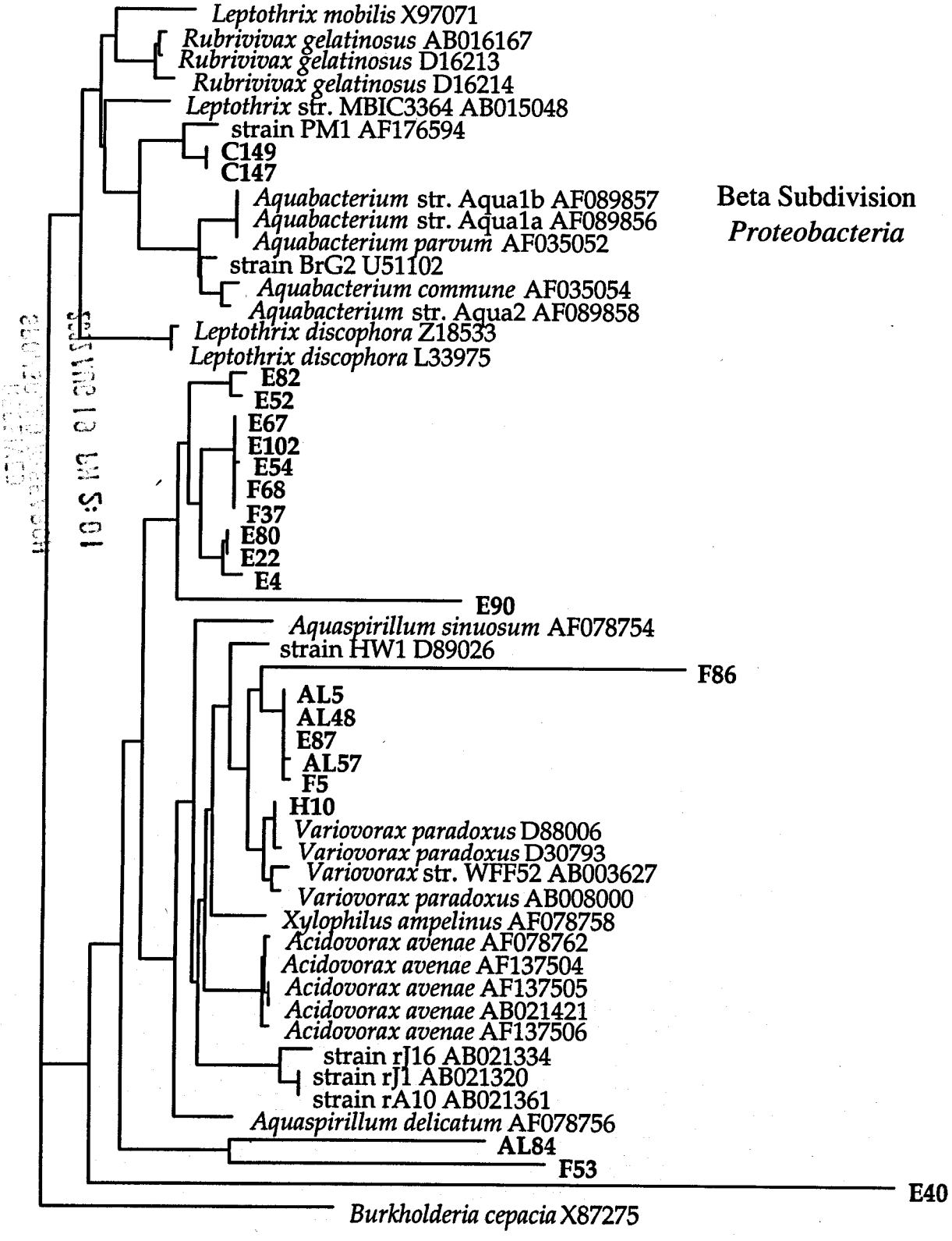
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C149
C147
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E102
E54
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E87
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F5
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strain rJ1 AB021320
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Aquaspirillum delicatum AF078756
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F53
E40
Burkholderia cepacia X87275
Beta Subdivision
Proteobacteria
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