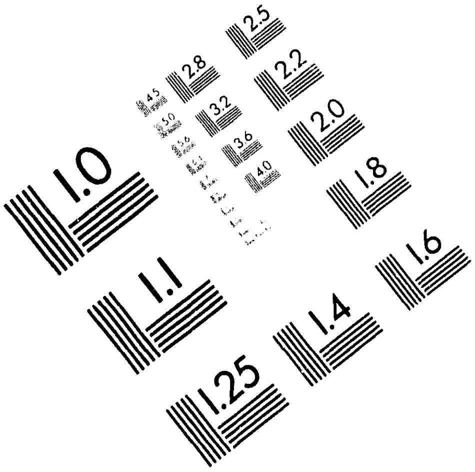
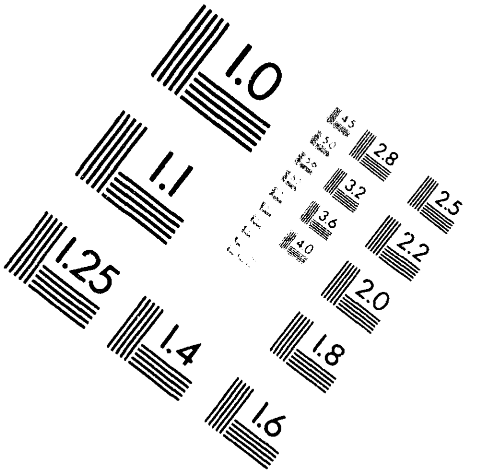
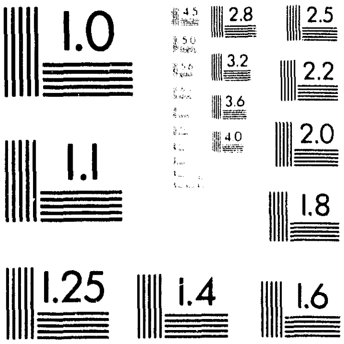


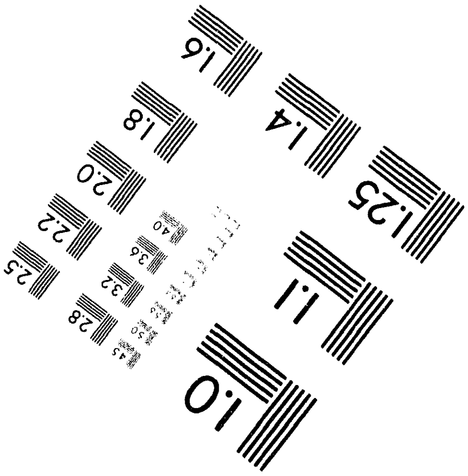
AIIM

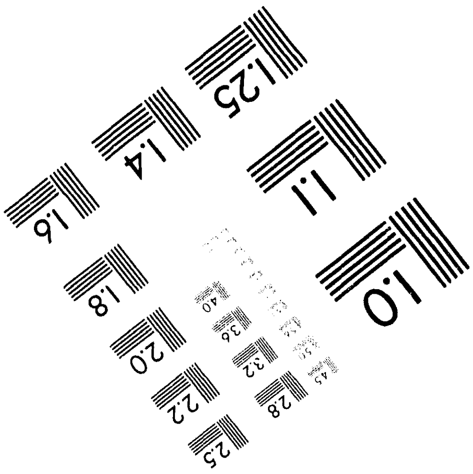
Association for Information and Image Management

1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202









MANUFACTURED TO AIIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

DOE/NV/10872--T110

3rd Quarterly Report (1/1/94 - 3/31/94)

1. Linda D. Stetzenbach, Harry Reid Center for Environmental Studies, University of Nevada, Las Vegas.

2. Identification of Subsurface Microorganisms at Yucca Mountain.

3. Bacteria isolated from ground water samples taken from 31 springs during 1993 were collected and processed according to procedures described in earlier reports. These procedures required aseptic collection of surface water samples in sterile screw-capped containers, transportation to the HRC microbiology laboratory, and culture by spread plating onto R2A medium. The isolates were further processed for identification using a gas chromatographic analysis of fatty acid methyl esters (FAME) extracted from cell membranes. This work generated a presumptive identification of 113 bacterial species distributed among 45 genera using a database obtained from Microbial ID, Inc., Newark, Delaware (MIDI).

A preliminary examination of the FAME data was accomplished using cluster analysis and principal component analysis software obtained from MIDI. Typically, bacterial strains that cluster at less than 10 Euclidian distance units have fatty acid patterns consistent among members of the same species. Thus an organism obtained from one source can be recognized if it is isolated again from the same or any other source. This makes it possible to track the distribution of organisms and monitor environmental conditions or fluid transport mechanisms. Microorganisms are seldom found as monocultures in natural environments. They are more likely to be closely associated with other genera with complementary metabolic requirements. An understanding of the indigenous microorganism population is useful in understanding subtle changes in the environment. However, classification of environmental organisms using traditional methods is not ideal because differentiation of species with small variations or genera with very similar taxonomic characteristics is beyond the capabilities of traditional microbiological methods.

However, the MIDI system provides a means to differentiate genera, species, subspecies, and strains. Information obtained by the MIDI system permits the generation of cluster relationships among unknown isolates and it can be used to quantitatively determine the degree of similarity of isolates. These features provide a means to compare unidentified bacteria with well characterized strains of known identity. A similarity index of 0.5 or greater is considered to be a very good match of an unknown organism with others in the MIDI database. Occasionally multiple identification matches are reported for a single isolate, each with a high similarity index. This is due

to similar organisms possessing nearly identical FAME profiles. Additional methods are needed to differentiate those organisms. Conversely, if the similarity index is very low or the profile is recorded as a "No Match", then the database library is lacking suitable representatives of the species in question. This is often the case when organisms from the natural environment are matched against libraries built with stock cultures.

To assist in identifying bacterial from environmental matrices, a new database (HRC-YM) is being constructed using waterborne bacteria isolated from spring waters sampled during this cooperative agreement. To construct the HRC-YM database, more than 650 bacteria isolated from the springs were first tested against the MIDI database. The MIDI database was originally developed with 645 unique entries representing 64 genera obtained from the stock collections of world wide contributors. The results of the spring water bacteria demonstrated a reasonably good name association for most organisms but the similarity index was often very low. Only a few of the more common genera demonstrated a similarity index of greater than 0.5 for a majority of the isolates classified within the given genus (Table 1). In addition, ninety isolates could not be identified and were each recorded as a "No Match" (data not shown).

The HRC-YM database is being developed using the organisms collected from waters sampled during this project and were processed according to the MIDI protocol. A group of bacteria identified by MIDI and having similar FAME patterns were selected as candidates for entry in the HRC-YM database using the identification listed in the MIDI database. Histograms of FAME features and their principal components were used to identify cohesive groups of specimens and eliminate outliers. After editing to select a homogenous set of organisms having similar characteristics, the prominent features of all members of the group were used to define the new entry. The HRC-YM database is being constructed with two or more specimens per entry and presently contains 72 entries representing 22 genera. To date, the isolates that had previously been tested with the MIDI database have been retested against the HRC-YM database. Results are shown in Table 1. The HRC-YM database has successfully recognized a large number of the test organisms and has named them with a high similarity index rating. To date, 433 species have been named and another 72 species that were previously unclassified have now been grouped into recognizable but unnamed subcategories. Approximately 150 organisms remain as "No Match." The HRC-YM database is more selective for many of the isolates used to develop it but because of the limited number of entries it is not yet as robust as the MIDI database. Expansion of the HRC-YM database by additional sampling and analysis will increase the utility of the new database and enhance identification of environmental organisms, resulting in a more complete characterization of the proposed Yucca Mountain site.

Table 1. Comparison of bacterial genera identified with the Microbial Identification (MIDI) data base and then processed with the Harry Reid Center-Yucca Mountain (HRC-YM) data base.

Bacterial genera identified with the MIDI data base (regardless of the similarity index value)				Values for the MIDI-named genera when processed with HRC-YM Data Base		
	n_a	# with index > 0.5	% with index > 0.5	n_b	# with index > 0.5	% with index > 0.5
Acidovorax	22	1	4.5	5	2	40.0
Aeromonas	2	0	0	19	12	63.2
Arthrobacter	12	4	33.3	9	3	33.3
Bacillus	45	5	11.1	8	6	75.0
Chromobacterium	9	8	88.9	5	4	80.0
Comamonas	15	2	13.3	2	2	100.0
Curtobacterium	24	6	25.0	16	13	81.3
Cytophaga	39	9	23.1	31	27	87.1
Enterobacter	18	13	72.2	6	4	66.7
Flavobacterium	6	0	0	2	2	100.0
Hydrogenophaga	34	2	5.9	25	6	24.0
Methylobacterium	9	1	11.1	4	4	100.0
Micrococcus	72	27	37.5	71	47	66.2
Pseudomonas	130	53	40.8	107	66	61.7
Serratia	8	4	50.0	2	2	100.0
Shewanella	3	0	0	4	3	75.0
Sphingobacterium	3	0	0	4	4	100.0
Sphingomonas	1	0	0	2	2	100.0
Staphylococcus	105	53	50.5	102	79	77.4
Variovorax	8	0	0	3	3	100.0
Vibrio	19	2	10.5	3	2	66.7
Xanthomonas	3	2	66.7	3	2	66.6

n_a = number of isolates named with the MIDI data base

n_b = number of MIDI-named isolates recognized with the HRC-YM data base.

DATE
FILMED
6/17/94

END