4th Quarterly Report

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 water samples collected in a series of ground water springs have been isolated, enumerated, and identified from twenty six sites. Ten sites were sampled in Death Valley, California and sixteen sites were sampled in Ash Meadows, Nevada. Replicate samples were collected and tested from four locations. All water samples were collected in conjunction with the HRC Chemistry group conducting ground water fingerprinting studies. The protocol for collection of samples, as described in the 3rd quarterly report, specified aseptic collection in sterile screw-capped containers and transportation on ice to the HRC microbiology laboratory. All samples were inoculated by spread plating onto R2A (Difco Laboratories, Detroit, MI) bacterial culture medium. The R2A plates were then incubated at 28° for 5-7 days and colonies were counted with the aid of a grid template and magnifying lens.

Table 1. Enumeration of bacteria as the number of colony forming units (CFU) per ml of water collected from sites at Death Valley National Monument, California.

Sample Date	Sample Site	Total CFU/ml
17 March '93	Grapevine	190
16 March '93	MacClean	7100
17 March '93	Mesquite	240
16 March '93	Navarres	1080
18 March '93	Saratoga	5550
17 March '93	Scotty's Castle	300
17 March '93	Surprise	1850
16 March '93	Texas Springs	1400
16 March '93	Travertine A	1200
16 March '93	Travertine B	880

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Table 2. Enumeration of bacteria as the number of colony forming units (CFU) isolated per ml of water from sites at Ash Meadows Wildlife Refuge, Nevada.

Sample Date	Sample Site	Total CFU/ml
25 Jan '93	Bradford	7040
15 Jan '93	Cold	100
25 Jan '93	Crystal	130
9 Jan '93	Devil's Hole, surface	50
9 Jan '93	Devil's Hole, 20' depth	130
9 Jan '93	Devil's Hole, 40' depth	90
9 Jan '93	Devil's Hole, 85' depth	130
15 Jan '93	Fairbanks	140
25 Jan '93	Forest	7200
15 Jan '93	Indian	100
25 Jan '93	Kingspool	7520
15 Jan '93	Longstreet	680
15 Jan '93	Marsh	70
11 Jan '93	Rogers 1	40
11 Jan '93	Rogers 2	50
11 Jan '93	School 1	6080
11 Jan '93	School 2	6000
15 Jan '93	Scruggs	230
11 Jan '93	Southeast Rocks	10
11 Jan '93	Southwest Rocks	110
11 Jan '93	Tubbs 1	3400
11 Jan '93	Tubbs 2	2000

Because environmental isolates may not grow well on nutrient-rich laboratory media, some of the isolates were first cultured on R2A agar and then subcultured to R2A and tryptic soy broth agar (TSBA, Difco) to determine if this procedure would improve the isolation rate. The results shown in Figure 1 indicate the mean percent recovery is about the same on R2A and TSBA. The two results correlate fairly well but individual values vary widely in mid-range, Figure 2. This may be due to the response of different species to the growth conditions and needs further investigation.

Figure 1. Comparison of percent recovery of organisms subcultured onto R2A and TSBA.

Figure 2. Regression plot of percent recovery of organisms plated onto two media.

Bacterial isolates were processed using the gas chromatographic (GC) based Microbial Identification System, MIDI (Microbial ID, INC., Newark, DE) method. According to the MIDI protocol, bacterial isolates were cultured on tryptic soy broth agar (TSBA, Difco) prior to harvesting. Previous work indicated that about 50 mg wet weight of cells were needed to produce sufficient fatty acid methyl ester peaks to identify an organism. However this rule of thumb seems to be somewhat dependent on the species and some organisms grow too slowly to accumulate that much mass within a reasonable time. A least squares regression plot of cell mass and peak area was performed for several species to determine the optimum mass for GC analysis. The results show the summed peak area counts for *Staphylococcus* sp. (Fig. 3), *Micrococcus* sp. (Fig. 4), and *Pseudomonas* sp. (Fig. 5) increase as cellular mass increases within the limits of ca. 10 mg to 60 mg wet weight. *Bacillus* sp. area versus mass plots are almost flat over a broad wet weight range (Fig. 6). Scatterplots of MIDI Similarity Index and mass do not show correlation (data not illustrated).

Identification of isolates using the MIDI system is listed in tables 3 and 4. Correlation of bacterial isolates with chemical parameters is pending.

Figure 3. Scatterplot of peak area counts (area) vs. wet weight (wetwt) for isolates identified as *Staphylococcus* sp. using the MIDI identification system.

Figure 4. Scatterplot of peak area counts (area) vs. wet weight (wetwt) for isolates identified as *Micrococcus* sp. using the MIDI identification system.

Figure 5. Scatterplot of peak area counts (area) vs. wet weight (wetwt) for isolates identified as *Pseudomonas* sp. using the MIDI identification system.

Figure 6. Scatterplot of peak area counts (area) vs. wet weight (wetwt) for isolates identified as *Bacillus* sp. using the MIDI identification system.

Table 3. Identification of isolates from the Death Valley National Monument.

SOURCE	SITE	GENUS1	SPECIES1	IDX1
DV	MACCLEAN	Bacillus	lentus	12
DV	MACCLEAN	Bacillus	pumilus sg B	801
DV	MACCLEAN	Corynebacterium	mediolamun	506
DV	MACCLEAN	Erwinia	salicis	828
DV	MACCLEAN	Hafnia	alvei	930
DV	MACCLEAN	Methylobacterium	zatmanii	442
DV	MACCLEAN	Micrococcus	roseus	614
DV	MACCLEAN	Micrococcus	roseus	640
DV	MACCLEAN	No Match		
DV	MACCLEAN	Serratia	fonticola	649

SOURCE	SITE	GENUS1	SPECIES1	IDX1
DV	MESQUITE	Acinetobacter	calcoaceticus	285
DV	MESQUITE	Micrococcus	lylae sg B	337
DV	MESQUITE	No Match		
DV	MESQUITE	Pseudomonas	pseudoalcaligenes	410
DV	MESQUITE	Pseudomonas	stutzeri	67
DV	MESQUITE	Pseudomonas	stutzeri	228
DV	MESQUITE	Pseudomonas	stutzeri	242
DV	MESQUITE	Pseudomonas	stutzeri	289
DV	MESQUITE	Pseudomonas	stutzeri	332
DV	MESQUITE	Pseudomonas	stutzeri	375
DV	MESQUITE	Pseudomonas	stutzeri	390
DV	MESQUITE	Pseudomonas	stutzeri	478
DV	MESQUITE	Pseudomonas	stutzeri	512
DV	MESQUITE	Pseudomonas	stutzeri	524

SOURCE	SITE	GENUS1	SPECIES1	IDX1
DV	NAVARRES	Arthrobacter	pascens	261
DV	NAVARRES	Bacillus	amyloliquefaciens	167
DV	NAVARRES	Bacillus	macroides	19
DV	NAVARRES	Micrococcus	luteus sg A	219
DV	NAVARRES	Micrococcus	luteus sg A	223
DV	NAVARRES	Micrococcus	luteus sg A	226
DV	NAVARRES	Micrococcus	luteus sg A	242
DV	NAVARRES	Micrococcus	luteus sg A	246
DV	NAVARRES	Micrococcus	luteus sg A	246
DV	NAVARRES	Micrococcus	roseus	211
DV	NAVARRES	Micrococcus	roseus	266
DV	NAVARRES	Micrococcus	roseus	314
DV	NAVARRES	Micrococcus	roseus	326
DV	NAVARRES	No Match		
DV	NAVARRES	No Match		
DV	NAVARRES	No Match		
DV	NAVARRES	No Match		

SOURCE	SITE	GENUS1	SPECIES1	IDX1
DV	SCOTTY'S	Agrobacterium	radiobacter	266
DV	SCOTTY'S	Curtobacterium	flaccumfaciens poin	540
DV	SCOTTY'S	Cytophaga	aquatis	464
DV	SCOTTY'S	Cytophaga	johnsonae	249
DV	SCOTTY'S	Micrococcus	luteus sg A	201
DV	SCOTTY'S	Micrococcus	luteus sg B	507
DV	SCOTTY'S	Micrococcus	lylae sg B	136
DV	SCOTTY'S	No Match		
DV	SCOTTY'S	Pseudomonas	syringae maculi	431

SOURCE	SITE	GENUS1	SPECIES1	IDX1
DV	TEXAS	Micrococcus	luteus sg A	209
DV	TEXAS	Micrococcus	lylae sg B	93
DV	TEXAS	Micrococcus	roseus	312
DV	TEXAS	Micrococcus	roseus	572
DV	TEXAS	Micrococcus	roseus	624
DV	TEXAS	Micrococcus	roseus	632
DV	TEXAS	Micrococcus	roseus	651
DV	TEXAS	Micrococcus	roseus	679
DV	TEXAS	Micrococcus	roseus	692
DV	TEXAS	Micrococcus	roseus	743
DV	TEXAS	Micrococcus	roseus	750
DV	TEXAS	Micrococcus	roseus	769
DV	TEXAS	Micrococcus	roseus	819
DV	TEXAS	No Match		

SOURCE	SITE	GENUS1	SPECIES1	IDX1
DV	TRAVA	Hydrogenophaga	palleronii	361
DV	TRAVA	Micrococcus	luteus sg A	110
DV	TRAVA	Micrococcus	luteus sg A	149
DV	TRAVA	Micrococcus	luteus sg A	198
DV	TRAVA	Micrococcus	roseus	467
DV	TRAVA	Micrococcus	roseus	526
DV	TRAVA	Micrococcus	roseus	570
DV	TRAVA	Micrococcus	roseus	662
DV	TRAVA	No Match		
DV	TRAVA	Staphylococcus	epidermidis	707
DV	TRAVA	Staphylococcus	epidermidis	712
DV	TRAVA	Staphylococcus	epidermidis	723
DV	TRAVA	Staphylococcus	epidermidis	741
DV	TRAVA	Staphylococcus	epidermidis	755
DV	TRAVA	Staphylococcus	epidermidis	756
DV	TRAVA	Staphylococcus	epidermidis	760
DV	TRAVA	Staphylococcus	epidermidis	777
DV	TRAVA	Staphylococcus	warneri	780
DV	TRAVA	Staphylococcus	warneri	791

[illegible]

Table 4. Identification of isolates from the Ash Meadows Wildlife Refuge.

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	BRADFORD	Alteromonas	haloplanktis	58
AM	BRADFORD	Alteromonas	haloplanktis	109
AM	BRADFORD	Alteromonas	haloplanktis	113
AM	BRADFORD	No Match		
AM	BRADFORD	No Match		
AM	BRADFORD	No Match		
AM	BRADFORD	No Match		
AM	BRADFORD	No Match		
AM	BRADFORD	No Match		
AM	BRADFORD	No Match		
AM	BRADFORD	Serratia	fonticola	797
AM	BRADFORD	Sphingobacterium	multivorum	84
AM	BRADFORD	Sphingobacterium	spiritivorum	37

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	COLD	Alteromonas	haloplanktis	92
AM	COLD	Arthrobacter	atrocyaneus	465
AM	COLD	Pseudomonas	vesicularis	136

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	CRYSTAL	Pseudomonas	diminuta	33
AM	CRYSTAL	Pseudomonas	vesicularis	209

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	DEVIL'S SURFAC	Enterococcus	gallinarum	855
AM	DEVIL'S SURFAC	Hydrogenophaga	pseudoflava	248

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	DEVIL'S 20'	Pseudomonas	diminuta	323
AM	DEVIL'S 20'	Pseudomonas	diminuta	880
AM	DEVIL'S 20'	Pseudomonas	saccharophila	403

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	DEVIL'S 40'	Clavibacter	michiganense in	18
AM	DEVIL'S 40'	Micrococcus	lylae sg B	91
AM	DEVIL'S 40'	Pseudomonas	diminuta	848

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	DEVIL'S 85'	Acinetobacter	lwoffii	412
AM	DEVIL'S 85'	Acinetobacter	lwoffii	572
AM	DEVIL'S 85'	No Match		
AM	DEVIL'S 85'	Pseudomonas	diminuta	850
AM	DEVIL'S 85'	Pseudomonas	diminuta	864
AM	DEVIL'S 85'	Pseudomonas	diminuta	870
AM	DEVIL'S 85'	Pseudomonas	saccharophila	550
AM	DEVIL'S 85'	Staphylococcus	hominis	755
AM	DEVIL'S 85'	Xanthomonas	maltophilia	557

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	FOREST	Hydrogenophaga	pseudoflava	80
AM	FOREST	Hydrogenophaga	pseudoflava	391
AM	FOREST	Methylobacterium	zatmanii	603
AM	FOREST	Micrococcus	luteus sg A	171
AM	FOREST	Micrococcus	varians	211
AM	FOREST	No Match		
AM	FOREST	No Match		
AM	FOREST	No Match		
AM	FOREST	Pseudomonas	vesicularis	163
AM	FOREST	Pseudomonas	vesicularis	684

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	INDIAN	Rhodococcus	equi sg B	205

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	KINGSPPOOL	Acidovorax	facilis	847
AM	KINGSPPOOL	Alcaligenes	piechaudii	173
AM	KINGSPPOOL	Alteromonas	haloplanktis	23
AM	KINGSPPOOL	Alteromonas	haloplanktis	27
AM	KINGSPPOOL	Alteromonas	haloplanktis	140
AM	KINGSPPOOL	Alteromonas	haloplanktis	220
AM	KINGSPPOOL	Alteromonas	haloplanktis	231
AM	KINGSPPOOL	Alteromonas	haloplanktis	235
AM	KINGSPPOOL	No Match		
AM	KINGSPPOOL	No Match		
AM	KINGSPPOOL	No Match		
AM	KINGSPPOOL	No Match		
AM	KINGSPPOOL	No Match		
AM	KINGSPPOOL	Pseudomonas	pseudoalcaligenes	215
AM	KINGSPPOOL	Pseudomonas	pseudoalcaligenes	307
AM	KINGSPPOOL	Sphingobacterium	multivorum	41
AM	KINGSPPOOL	Weeksella	virosa	45

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	LONGSTREET	Aeromonas	hydrophila	778
AM	LONGSTREET	Bacillus	coagulans sg B	222
AM	LONGSTREET	Cytophaga	johnsonae	14
AM	LONGSTREET	Cytophaga	johnsonae	57
AM	LONGSTREET	Enterobacter	agglomerans	360
AM	LONGSTREET	Enterobacter	agglomerans	395
AM	LONGSTREET	Enterobacter	agglomerans	431
AM	LONGSTREET	Methylobacterium	zatmanii	472
AM	LONGSTREET	Shewanella	putrefaciens	50
AM	LONGSTREET	Xanthobacter	flavus	307

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	MARSH	Flavobacterium	resinovorum	460
AM	MARSH	No Match		

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	ROGERS	Listonella	anguillarum	161
AM	ROGERS	Pseudomonas	coronafaciens	388

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	SCHOOL1	Alteromonas	haloplanktis	120
AM	SCHOOL1	Alteromonas	haloplanktis	251
AM	SCHOOL1	Arthrobacter	atrocyaneus	450
AM	SCHOOL1	Chromobacterium	violaceum	537
AM	SCHOOL1	Chromobacterium	violaceum	576
AM	SCHOOL1	Micrococcus	luteus sg A	482
AM	SCHOOL1	No Match		
AM	SCHOOL1	Pseudomonas	alcaligenes	241
AM	SCHOOL1	Pseudomonas	coronafaciens	252
AM	SCHOOL1	Pseudomonas	pseudoalcaligenes	200
AM	SCHOOL1	Pseudomonas	stutzeri	663
AM	SCHOOL1	Pseudomonas	stutzeri	776
AM	SCHOOL1	Pseudomonas	stutzeri	838

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	SCHOOL2	Chromobacterium	violaceum	498
AM	SCHOOL2	Chromobacterium	violaceum	563
AM	SCHOOL2	No Match		
AM	SCHOOL2	No Match		
AM	SCHOOL2	Pseudomonas	alcaligenes	629
AM	SCHOOL2	Pseudomonas	coronafaciens	533
AM	SCHOOL2	Pseudomonas	coronafaciens	578
AM	SCHOOL2	Pseudomonas	stutzeri	872
AM	SCHOOL2	Pseudomonas	stutzeri	889
AM	SCHOOL2	Pseudomonas	stutzeri	922
AM	SCHOOL2	Pseudomonas	syringae maculi	542

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	SCRUGGS	Agrobacterium	radiobacter	426
AM	SCRUGGS	Micrococcus	luteus sg A	32
AM	SCRUGGS	Micrococcus	lylae sg B	27
AM	SCRUGGS	No Match		
AM	SCRUGGS	Sphingomonas	paucimobilis	73

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	SW ROCKS	No Match		
AM	SW ROCKS	Pseudomonas	vesicularis	158
AM	SW ROCKS	Vibrio	fluvialis	18

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	TUBBS1	Alteromonas	haloplanktis	86
AM	TUBBS1	Ctyophaga	johnsonae	98
AM	TUBBS1	No Match		
AM	TUBBS1	No Match		
AM	TUBBS1	No Match		
AM	TUBBS1	Pseudomonas	syringae maculi	913
AM	TUBBS1	Pseudomonas	syringae maculi	913

SOURCE	SITE	GENUS1	SPECIES1	IDX1
AM	TUBBS2	Curtobacterium	flaccumfacien f	33
AM	TUBBS2	Curtobacterium	flaccumfacien poin	719
AM	TUBBS2	No Match		
AM	TUBBS2	Pseudomonas	syringae maculi	178
AM	TUBBS2	Staphylococcus	xylosus	141

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