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Quarterly Report (7/01/95 - 9/30/95)

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2. Identification of Subsurface Microorganisms at Yucca Mountain.

3. More than 1100 bacterial isolates were obtained over a two year period from 31 springs in a region along the southern boarder of California and Nevada. Water samples were collected from 17 springs in Ash Meadows National Wildlife Refuge and 14 springs in Death Valley National Park. Bacteria isolated from these samples were processed according to procedures described in earlier quarterly reports and were subjected to extraction and gas chromatography to determine the cellular fatty acid profile of each isolate. Fatty acid methyl esters (FAME) extracted from cell membranes were separated and classified using the Hewlett Packard gas chromatographic system described in earlier quarterly reports. The FAME profiles of each isolate were then subjected to cluster analysis by the unweighted pair-group method using arithmetic averages as provided by software obtained from Microbial ID, Inc., Newark, Delaware (MIDI).

During this quarter the relatedness of FAME patterns of bacterial isolates were examined at the genus level by counting the number of clusters produced in a MIDI dendrogram at a Euclidian distance of 25. This information was then used to determine microbiological relationships among springs. A total of 544 bacterial isolates from Ash Meadows were evaluated. Two hundred seventy nine of those were collected during 1993 and 265 were obtained during 1994. A total of 568 samples from Death Valley were collected and analyzed in two groups: a 265 member group of samples taken from January to June of 1993 and a second group of 303 samples obtained during late autumn sampling trips made in 1993 and 1994. Because of computer program limitations, cluster analysis was performed separately on each group. In each set of data, clusters were designated in succession by a letter of the alphabet from A to Z . However, a cluster designated by a specific letter in one data set does not necessarily correspond to a similarly designated cluster in another data set.

The data obtained from the 1993 Ash Meadows samples show that the highest number of clusters, indicating the greatest diversity of bacterial genera, were found in Bradford and Forest springs (Table 1). These springs are located within 2 km of each other in the central region of Ash Meadows. Both springs contain small fish and have tall, dense vegetation surrounding the pools formed from the groundwater outflows. Other central Ash Meadows springs (Devils Hole, Kings Pool, Tubbs, Cold, and Point of Rocks) had a decreasing number of clusters indicating a decreasing number of genera isolated (Table 1). Table 2 lists the number of genera common to both members of a pair of springs. By examining various combinations of spring pairs it is evident that many of the Ash

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Meadows springs have common clusters of bacterial genera. Bradford and Forest shared common genera with all of the other Ash Meadows springs sampled and greater than half of the clusters were shared with Devils Hole, Fairbanks, Kings Pool, Tubbs, Point of Rocks and Rogers. This may indicate a relatedness of the waters in these springs. Marsh and Indian springs had the lowest number of isolates and, consequently, the least number of similar clusters when paired with other springs.

The data collected from Ash Meadows in 1994 closely resembled the 1993 data. Bradford spring again produced the largest diversity of genera followed closely by Crystal and Kings Pool (Table 3). These three springs shared a high proportion of genera in common. They also show a relatedness with the northern Ash Meadows springs of Fairbanks, Longstreet and Rogers (Table 4). Jackrabbit, Scruggs and Cold springs had similar numbers of genera isolated although the total number of genera was less than previously discussed springs. Big spring had the least number of genera in common with other springs. Forest, Tubbs, Marsh and Indian springs were not sampled during the 1994 sampling period.

The January to June set of 1993 Death Valley samples produced 19 clusters at the genus level with the most diversity found in McLean spring followed by Scotty's Castle, Travertine B and Texas (Table 5). Briar Patch, Mesquite, Nevares, Saratoga and Woodcamp are among the springs which produced the least number of genera clusters. The Scotty's Castle spring contained organisms which were the same genus as bacteria isolated from Surprise, Briar Patch, and Woodcamp (Table 6). All of these springs are located in the northeastern section of the Death Valley region. Scotty's Castle also had 5 genera in common with Travertine B, a centrally located spring. Table 6 shows that the central Death Valley group of springs (Texas, Travertine A, Travertine B and Nevares) have some bacterial genera in common with each other but share few genera with the southern spring (Saratoga).

A similar yield of 19 clusters of different genera was obtained from the set of Death Valley samples collected from the fall of 1993 through 1994. However the order of prevalence of cluster types varied from the earlier data. For example, a greater diversity of bacterial genera was found at Mesquite and Surprise springs during the second sampling period (Table 7) than in the earlier sampling (Table 5). Table 8 shows a group of clusters common to pairs of springs in northern Death Valley (Mesquite, Surprise, Grapevine, Scotty's Castle springs). Travertine A, Travertine B, Upper Grapevine and Saratoga also show a number of genera shared with the northern group and among themselves. Nevares and Texas springs yielded very few isolates in this data set and showed few genus level relationships to other springs in the data set.

Table 1. Number of FAME clusters at the genus level obtained from bacteria isolated from Ash Meadows springs in 1993 (Brad = Bradford; Fors = Forest; DH = Devils Hole; Fair = Fairbanks; King = Kings Pool; Tub = Tubbs; Cold = Cold; Rock = Point of Rocks; Scho = School; Long = Longstreet; Rog = Rogers; Big = Big; Crys = Crystal; Scr = Scruggs; Jack = Jackrabbit; Indi = Indian; Mars = Marsh; Type = alphabetical designations for clusters at the genus level of relatedness; Sum = the number of springs which contain a specific cluster type; Count = the number of genera clusters found in each spring).

Type	Brad	Fors	DH	Fair	King	Tub	Cold	Roc	Sch	Lon	Rog	Big	Crys	Scr	Jack	Indi	Mar	Sum
S	1	1	1	1	1	1	1		1	1	1	1	1		1			13
Q	1	1	1	1	1	1	1		1		1	1	1		1			12
J		1	1	1	1		1	1				1	1			1		9
K	1	1	1	1			1				1		1	1			1	9
L	1	1	1		1	1		1	1		1	1						9
R	1	1		1	1	1	1	1			1							8
A	1	1		1				1		1				1		1		7
P	1	1			1			1	1		1	1						7
H	1	1	1		1	1				1								6
B				1		1	1		1	1								5
C	1	1				1		1						1				5
G	1		1	1					1					1				5
I	1	1		1	1			1										5
O	1				1	1				1								4
F				1		1	1											3
D			1												1			2
E			1							1								2
M			1						1									2
N		1					1											2
Count	12	12	10	10	9	9	8	7	7	6	6	5	4	4	3	2	1	115

Table 2. Number of FAME-derived bacterial genera clusters common to each pair of Ash Meadows springs sampled in 1993 (Brad = Bradford; Fors = Forest; DH = Devils Hole; Fair = Fairbanks; King = Kings Pool; Tub = Tubbs; Cold = Cold; Rock = Point of Rocks; Scho = School; Long = Longstreet; Rog = Rogers; Big = Big; Crys = Crystal; Scr = Scruggs; Jack = Jackrabbit; Indi = Indian; Mars = Marsh).

	Fors	DH	Fair	King	Tub	Cold	Rock	Scho	Long	Rog	Big	Cryst	Scr	Jack	Indi	Mars
Brad	10	6	7	8	7	4	6	5	4	6	4	3	4	2	1	1
Fors		6	7	8	6	6	7	4	3	6	5	4	3	2	2	1
DH			5	5	4	4	2	5	3	4	4	4	2	3	1	1
Fair				5	5	7	4	4	3	4	3	4	3	2	2	1
King					6	4	5	4	3	5	5	3	0	2	1	0
Tub						5	3	4	4	4	3	2	1	2	0	0
Cold							2	3	2	4	3	4	1	2	1	1
Rock								2	1	3	3	1	2	0	2	0
Scho									2	4	4	2	1	2	0	0
Long										1	1	1	1	1	1	0
Rog											4	3	1	2	0	1
Big												3	0	2	1	0
Cryst													1	2	1	1
Scr														0	1	1
Jack															0	0
Indi																0

Table 3. Number of FAME clusters at the genus level obtained from bacteria isolated from Ash Meadows springs in 1994 (Brad = Bradford; Crys = Crystal; King = Kings Pool; Fair = Fairbanks; Long = Longstreet; Jack = Jackrabbit; Rog = Rogers; Scr = Scruggs; Cold = Cold; Rock = Point of Rocks; Big = Big; Type = alphabetical designations for clusters at the genus level of relatedness; Sum = the number of springs which contain a specific cluster type; Count = the number of genera clusters found in each spring).

Type	Brad	Crys	King	Fair	Long	Jack	Rog	Scr	Cold	Rock	Big	Sum
H	1	1	1	1	1	1	1	1	1		1	10
A	1	1	1	1			1	1	1	1		8
C	1	1	1		1	1	1	1		1		8
E	1	1	1	1	1	1		1	1			8
I	1	1	1	1	1		1			1	1	8
B		1	1	1	1			1	1			6
F	1		1			1	1					4
G	1	1	1	1								4
K	1	1		1	1							4
L	1		1	1	1							4
J		1			1					1		3
D	1					1						2
M											1	1
Count	10	9	9	8	8	5	5	5	4	4	3	70

Table 4. Number of FAME-derived bacterial genera clusters common to each pair of springs located at the intersection of the horizontal and vertical list of springs sampled in 1994 (Brad = Bradford; Crys = Crystal; King = Kings Pool; Fair = Fairbanks; Long = Longstreet; Jack = Jackrabbit; Rog = Rogers; Scr = Scruggs; Cold = Cold; Rock = Point of Rocks; Big = Big).

	Brad	Crys	King	Fair	Long	Jack	Rog	Scr	Cold	Rock	Big
Brad		7	6	7	6	5	5	4	3	3	2
Crys			7	7	7	3	4	5	4	3	2
King				7	6	4	5	5	4	3	2
Fair					6	2	3	4	4	2	2
Long						3	3	4	3	3	2
Jack							3	3	2	1	1
Rog								3	2	3	2
Scr									4	2	1
Cold										1	1
Rock											1

Table 5. Number of FAME clusters at the genus level obtained from bacteria isolated from Death Valley springs from January to June 1993 (Mcl = McLean; Scot = Scotty's Castle; Trav B = Travertine B; Tex = Texas; Brit = Briar Top; Sur = Surprise; Trav A = Travertine A; Briar = Briar Patch; Mes = Mesquite; Nev = Nevares; Sara = Saratoga; Wood = Woodcamp; Type = alphabetical designations for clusters at the genus level of relatedness; Sum = the number of springs which contain a specific cluster type; Count = the number of genera clusters found in each spring).

Type	Mcl	Scot	TravB	Tex	Brit	Sur	TravA	Briar	Mes	Nev	Sara	Wood	Sum
I	1	1	1	1			1	1		1		1	8
B	1	1			1	1	1		1		1		7
J	1		1	1			1			1			5
M	1	1	1		1							1	5
Q	1	1	1			1		1					5
A	1			1					1		1		4
E	1	1	1									1	4
G	1	1				1			1				4
L	1	1	1							1			4
C	1				1						1		3
N	1			1	1								3
O	1			1			1						3
F					1	1							2
H			1	1									2
K		1						1					2
R	1		1										2
S			1	1									2
D		1											1
P	1												1
Count	14	9	9	7	5	4	4	3	3	3	3	3	67

Table 6. Number of FAME-derived bacterial genera clusters common to each pair of Death Valley springs sampled from January to June 1993 (Mcl = McLean; Scot = Scotty's Castle; Trav B = Travertine B; Tex = Texas; Brit = Briar Top; Sur = Surprise; Trav A = Travertine A; Briar = Briar Patch; Mes = Mesquite; Nev = Nevares; Sara = Saratoga; Wood = Woodcamp).

	Scot	TravB	Tex	Brit	Sur	TravA	Briar	Mes	Nev	Sara	Wood
Mcl	7	7	5	4	3	4	2	3	3	3	3
Scot		5	1	2	3	2	3	2	2	1	3
TravB			4	1	1	2	2	0	3	0	3
Tex				1	0	3	1	1	2	1	1
Brit					2	1	0	1	0	2	1
Sur						1	1	2	0	1	0
TravA							1	1	2	1	1
Briar								0	1	0	1
Mes									0	2	0
Nev										0	1
Sara											0

Table 7. Number of FAME clusters at the genus level obtained from bacteria isolated from Death Valley springs from late 1993 to the end of 1994 (Mes = Mesquite; Sur = Surprise; Grape = Grapevine; Scot = Scotty's Castle; Trav B = Travertine B; Trav A = Travertine A; UpGr = Upper Grapevine; Mcl = McLean; Sara = Saratoga; Nev = Nevares; Tex = Texas; Type = alphabetical designations for clusters at the genus level of relatedness; Sum = the number of springs which contain a specific cluster type; Count = the number of genera clusters found in each spring).

Type	Mesq	Sur	Grape	Scot	TravB	TravA	UpGr	Mcl	Sara	Nev	Tex	Sum
A	1	1	1	1	1	1	1	1	1			9
C	1	1	1	1		1	1		1	1		8
F	1	1	1		1	1	1	1				7
E		1	1	1		1	1		1			6
D	1		1	1		1	1					5
I	1		1					1			1	4
N	1	1		1	1							4
P		1		1	1				1			4
Q	1	1	1			1						4
B	1	1						1				3
J		1	1		1							3
O				1	1		1					3
H				1	1							2
K			1							1		2
L	1										1	2
M		1		1								2
G								1				1
R										1		1
S	1											1
Count	10	10	9	9	7	6	6	5	4	3	2	71

Table 8. Number of FAME-derived bacterial genera clusters common to each pair of Death Valley springs sampled from late 1993 to the end of 1994 (Mes = Mesquite; Sur = Surprise; Grape = Grapevine; Scot = Scotty's Castle; Trav B = Travertine B; Trav A = Travertine A; UpGr = Upper Grapevine; Mcl = McLean; Sara = Saratoga; Nev = Nevares; Tex = Texas).

	Mesq	Sur	Grape	Scot	TravB	TravA	UpGr	Mcl	Sara	Nev	Tex
Mesq		6	6	4	3	5	4	4	2	1	2
Sur			6	6	5	5	4	3	4	1	0
Grape				4	3	6	5	3	3	2	1
Scot					5	4	5	1	4	1	0
TravB						2	3	2	2	0	0
TravA							5	2	3	1	0
UpGr								2	3	1	0
Mcl									1	0	1
Sara										1	0
Nev											0
Tex											