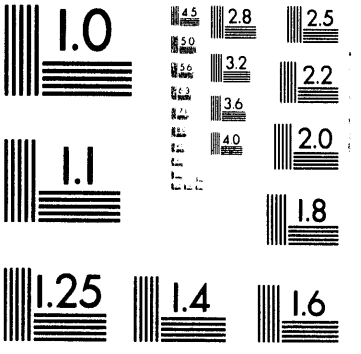
1.0
4.5
2.8
2.5
5.0
3.2
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1.25
1.4
1.6

1 of 1

DOE/NV/10872--T94

2nd Quarterly Report (10/1/93 - 12/31/93)

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 from 31 springs during 1993 were processed according to the protocol described in the April 1993 quarterly report. This protocol specified aseptic collection in sterile screw-capped containers, transportation on ice to the HRC microbiology laboratory, and culture by spread plating onto R2A medium. The isolates were further processed for identification using a gas chromatographic (GC) method based on the Microbial Identification System (MIDI) software obtained from Microbial ID, INC., Newark, DE. This work generated a presumptive identification of 113 bacterial species distributed among 45 genera.

The primary effort of this past quarter was to develop a procedure where accumulated data files could be evaluated to determine the naming consistency and inter-relationships of the various species which have been identified by the MIDI system. This involved a series of steps, including the clustering of similarly named organisms in a dendrogram format to determine how closely similarly named isolates are related (Figure 1). The experience of other researchers using the MIDI system has shown that clusters which are joined at a Euclidian distance of 10 or less belong to the same species. Strains which are very similar cluster at less than 6 Euclidian units and clusters below two units have nearly identical fatty acid patterns. When the dendrograms derived from the springs were scrutinized, some organisms were found which did not match the pattern of their named group. Then a decision was made whether to rename the isolates and exclude them from the group or redefine the group. This decision was assisted by plotting the principal components derived from an analysis of the fatty acid composition of members of the genus. This is illustrated in Figure 2 by an analysis of members of the genus, *Micrococcus,* which were isolated from the springs. Each species can be examined by the same procedure to determine group homogeneity (Figures 3-5). In these 2-dimensional plots members of the same species are roughly bounded by a box of 100 squared units while closely related strains are grouped more tightly together. The 2-dimensional plot of isolates of *Micrococcus luteus* demonstrates the presence of three identifiable sub-species (Figure 6).

After group clusters are defined, members are used to create a new library of micro-organisms unique to the biome of interest. The process of library generation requires that several preliminary steps have been accomplished. These include: uniform sample preparation, adequate chromatographic separation, proper calibration leading to reliable peak identification and quantification, and accumulation of refined data which can be

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organized into taxonomic subgroups. The entire matrix of fatty acid methyl esters derived from a selected subgroup are considered as training parameters for a prospective library entry (Figure 7). However some of the fatty acids, also called "features", are found only in a small percentage of the organisms that make up a taxonomic subgroup. These atypical fatty acids are not used for defining the profile of the new library entry. The remaining fatty acids can be individually plotted on a histogram to be examined for outliers and edited accordingly. The histogram can be based on the principle components of a feature in question or on the distance of each sample from the n-dimensional mean of all of the samples (Figures 8-9). The histogram can also serve to illustrate logical subdivisions that may represent different strains within a taxonomic subgroup as shown in Figure 10.

Once all the feature and histogram editing has been accomplished, the remaining fatty acid profile is named and added to the new library. The library entries can then be used as a basis for comparison and evaluation of the degree of similarity with other organisms. During the next quarter the necessary editing for a number of the more common isolates will be accomplished and the profiles will be added to one or more new libraries.

4. n/a

5. n/a

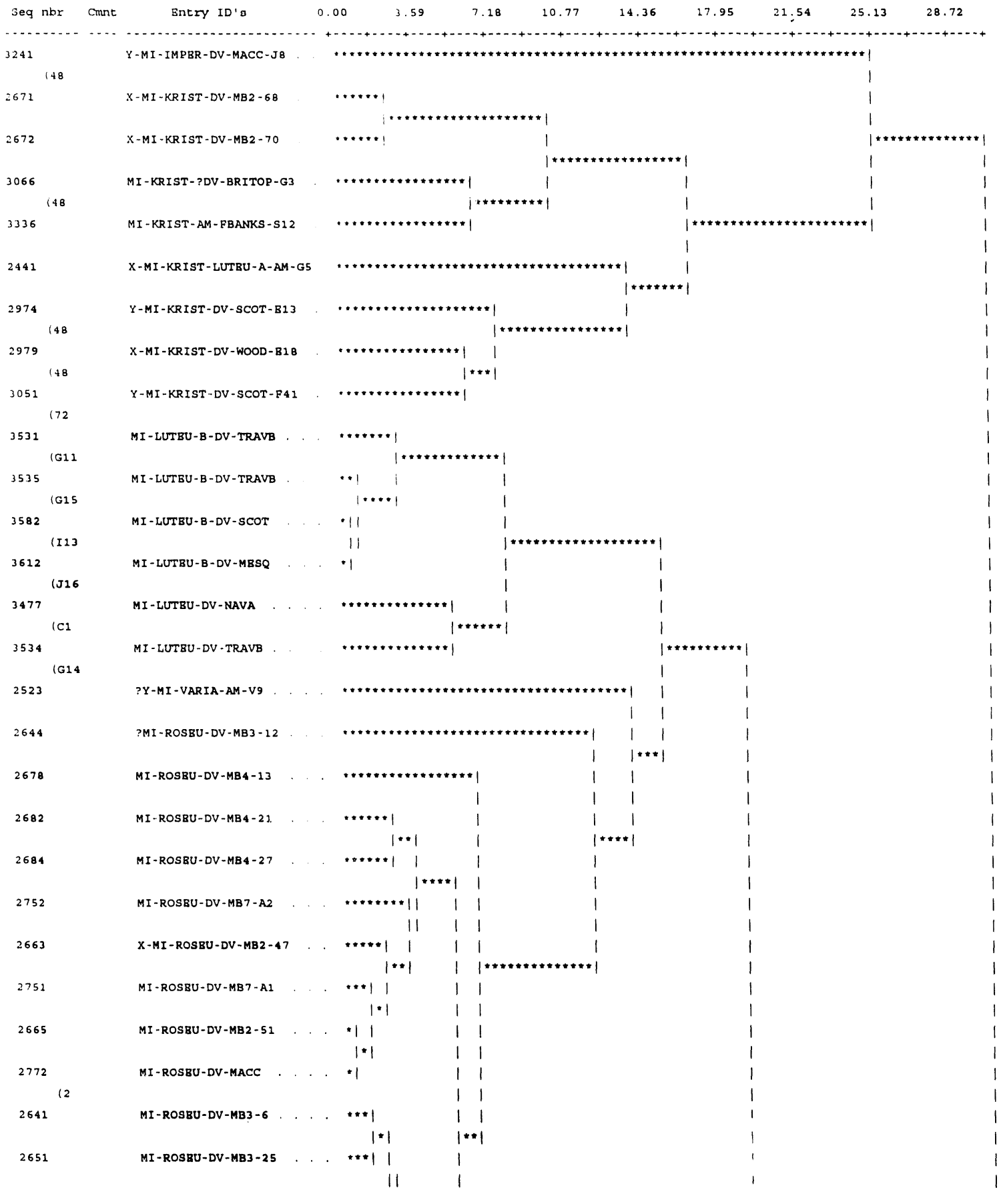
6. n/a

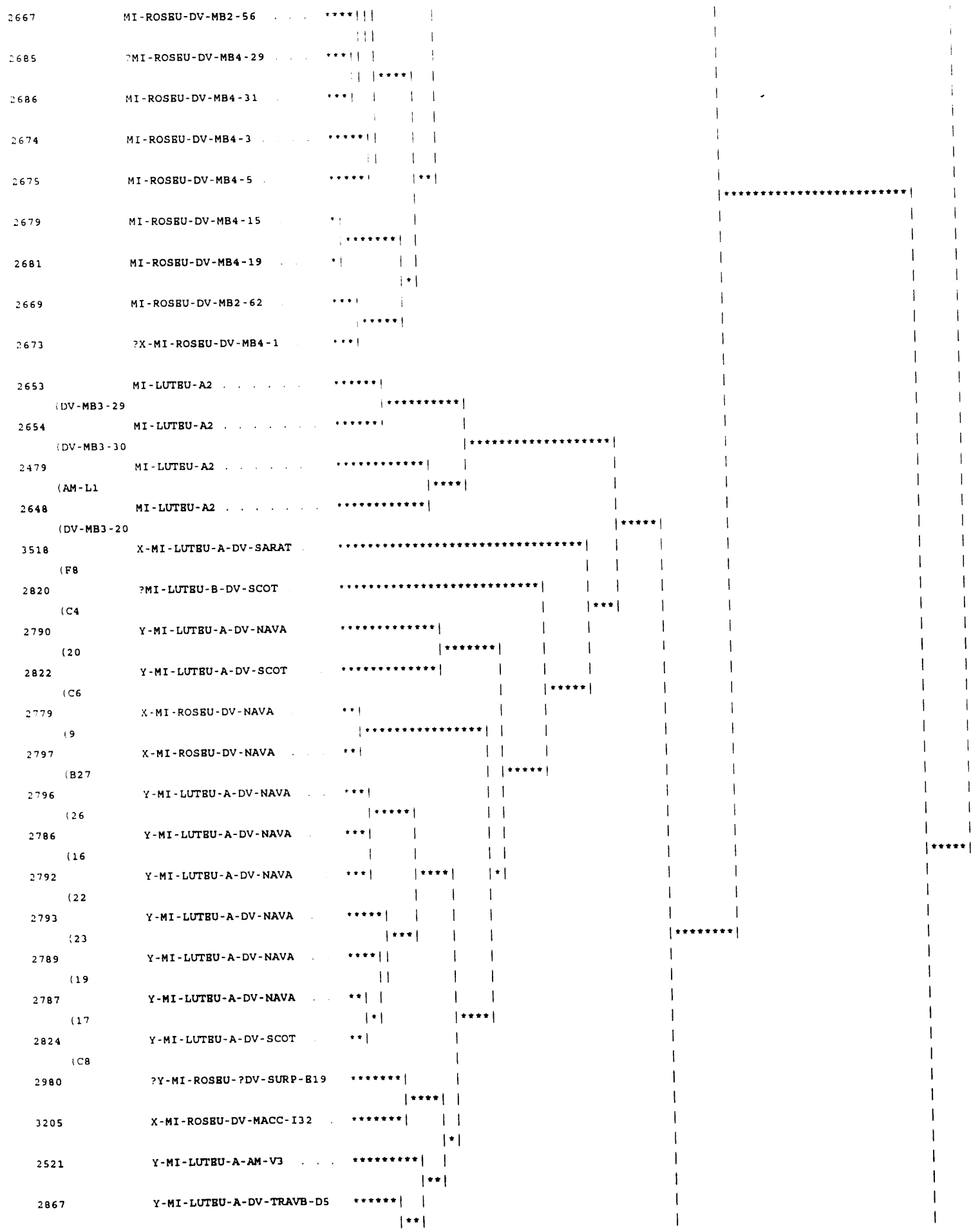
7. Enclosed.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Euclidian Distance




2667 MI-ROSEU-DV-MB2-56
2685 ?MI-ROSEU-DV-MB4-29
2686 MI-ROSEU-DV-MB4-31
2674 MI-ROSEU-DV-MB4-3
2675 MI-ROSEU-DV-MB4-5
2679 MI-ROSEU-DV-MB4-15
2681 MI-ROSEU-DV-MB4-19
2669 MI-ROSEU-DV-MB2-62
2673 ?X-MI-ROSEU-DV-MB4-1
2653 MI-LUTEU-A2
(DV-MB3-29
2654 MI-LUTEU-A2
(DV-MB3-30
2479 MI-LUTEU-A2
(AM-L1
2648 MI-LUTEU-A2
(DV-MB3-20
3518 X-MI-LUTEU-A-DV-SARAT
(F8
2820 ?MI-LUTEU-B-DV-SCOT
(C4
2790 Y-MI-LUTEU-A-DV-NAVA
(20
2822 Y-MI-LUTEU-A-DV-SCOT
(C6
2779 X-MI-ROSEU-DV-NAVA
(9
2797 X-MI-ROSEU-DV-NAVA
(B27
2796 Y-MI-LUTEU-A-DV-NAVA
(26
2786 Y-MI-LUTEU-A-DV-NAVA
(16
2792 Y-MI-LUTEU-A-DV-NAVA
(22
2793 Y-MI-LUTEU-A-DV-NAVA
(23
2789 Y-MI-LUTEU-A-DV-NAVA
(19
2787 Y-MI-LUTEU-A-DV-NAVA
(17
2824 Y-MI-LUTEU-A-DV-SCOT
(C8
2980 ?Y-MI-ROSEU-?DV-SURP-E19
3205 X-MI-ROSEU-DV-MACC-I32
2521 Y-MI-LUTEU-A-AM-V3
2867 Y-MI-LUTEU-A-DV-TRAVB-D5

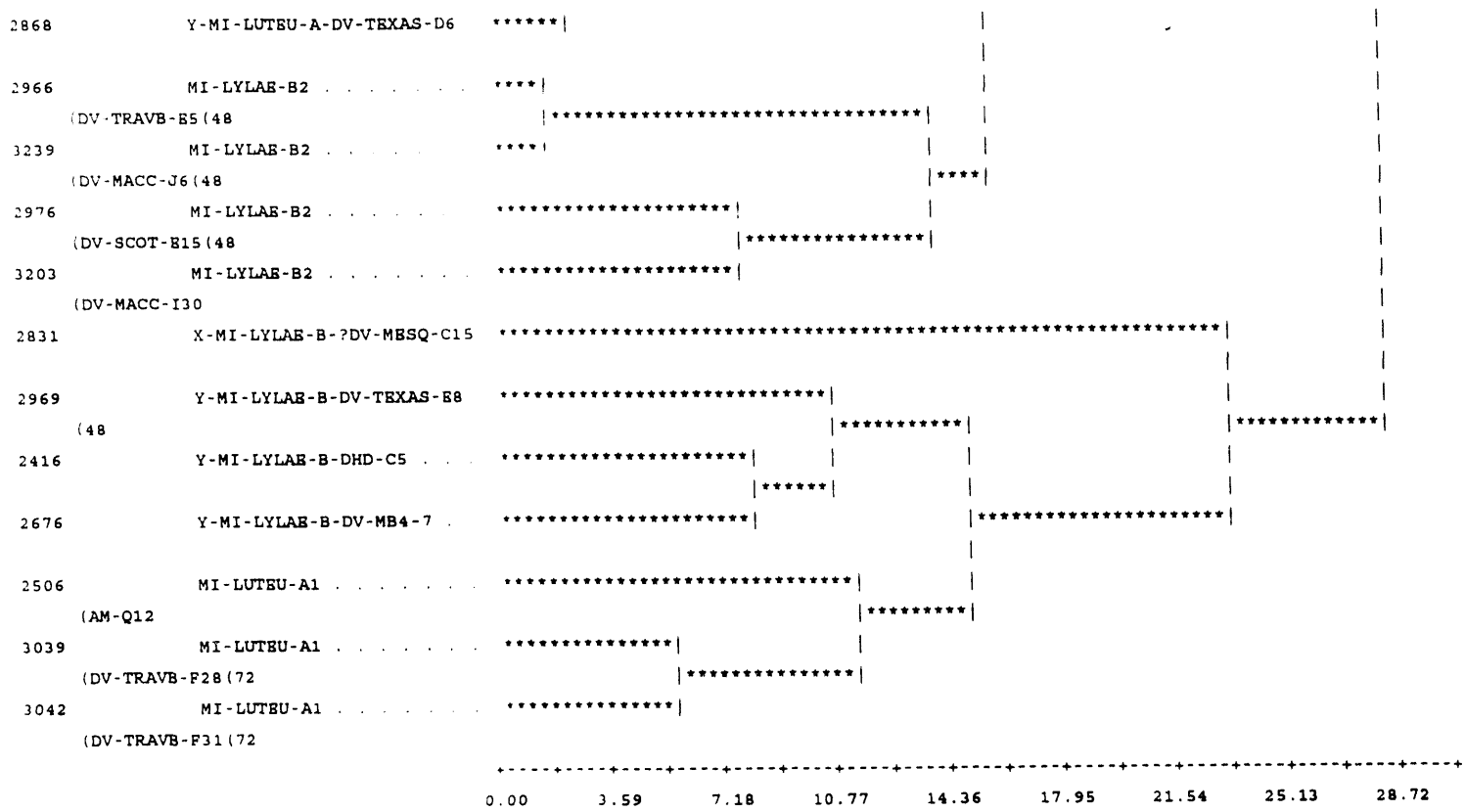


Figure 1. Dendrogram of *M. icrococcus* isolates obtained from spring water samples.

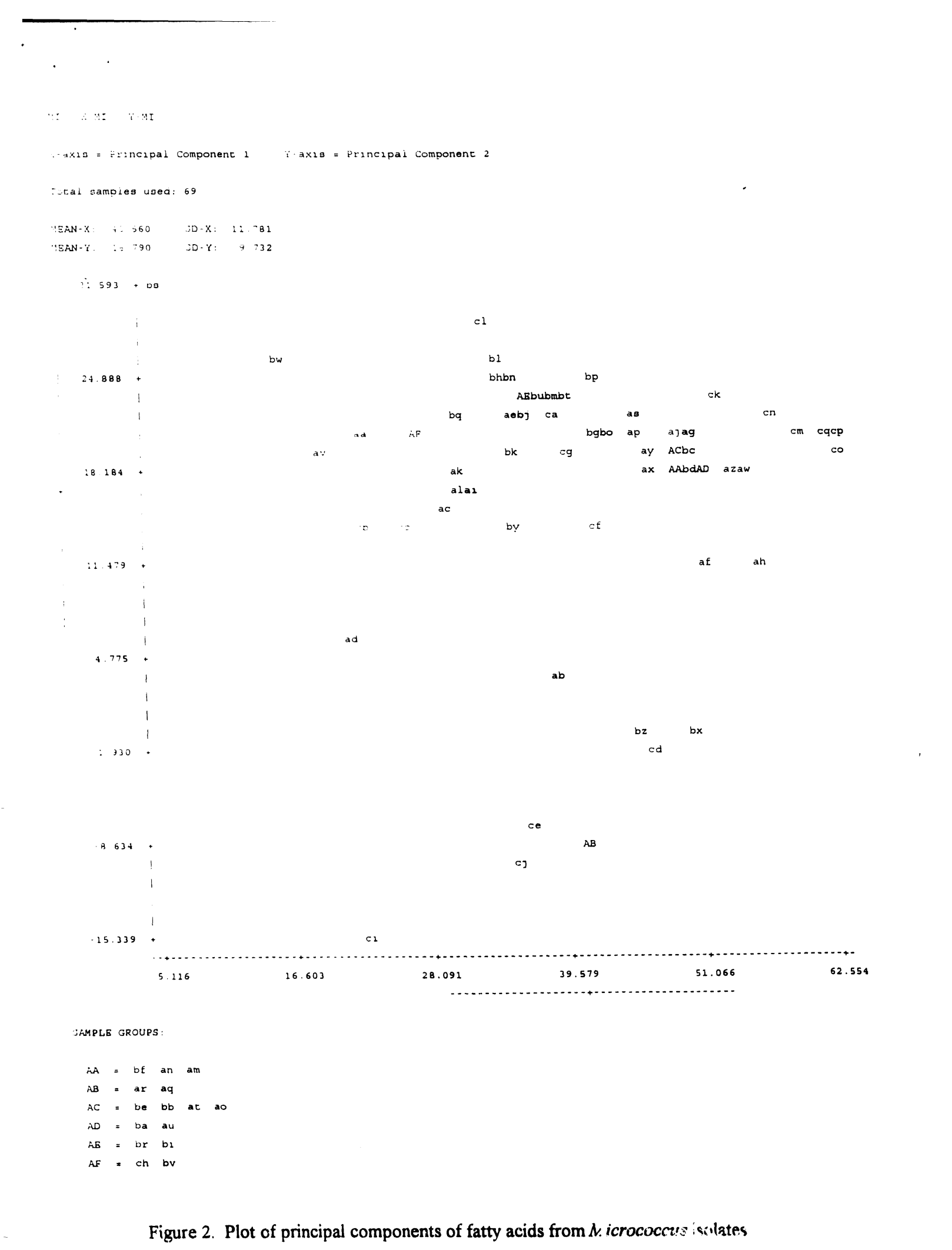


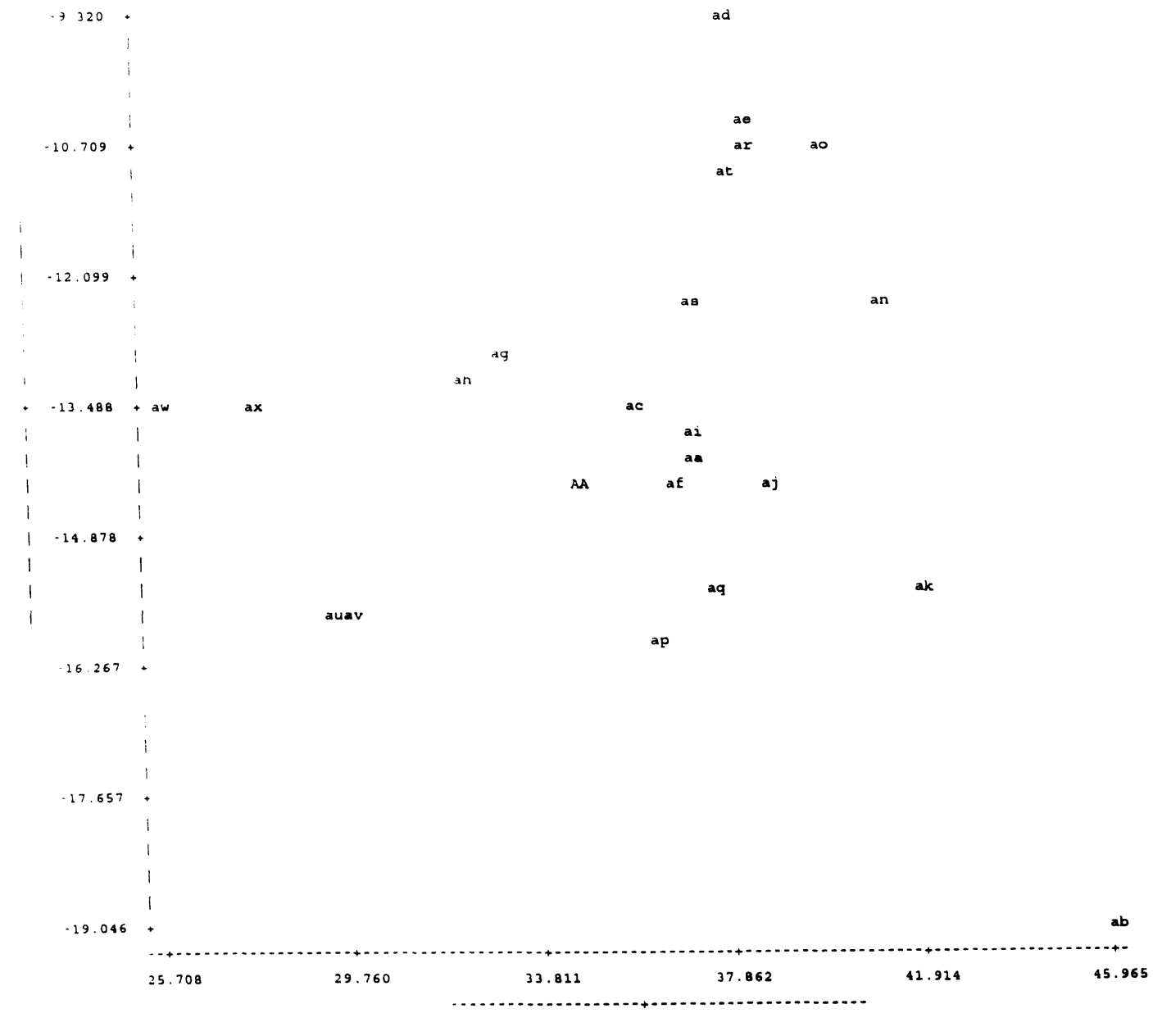
Figure 2. Plot of principal components of fatty acids from *M. icrococcus* isolates

MI-ROSEU X-MI-ROSEU Y-MI-ROSEU

X-axis = Principal Component 1 Y-axis = Principal Component 2

Total samples used: 24

MEAN-X: 15.996 SD-X: 4.605
MEAN-Y: -13.588 SD-Y: 2.158



SAMPLE GROUPS:

AA = am al

Figure 3. Plot of principal components of fatty acids from isolates of *M. icrococcus roseus.*

MI-KRIST X-MI-KRIST Y-MI-KRIST

X-axis = Principal Component 1 Y-axis = Principal Component 2

Total samples used: 8

MEAN-X: -19.190 SD-X: 8.305
MEAN-Y: -14.004 SD-Y: 4.157

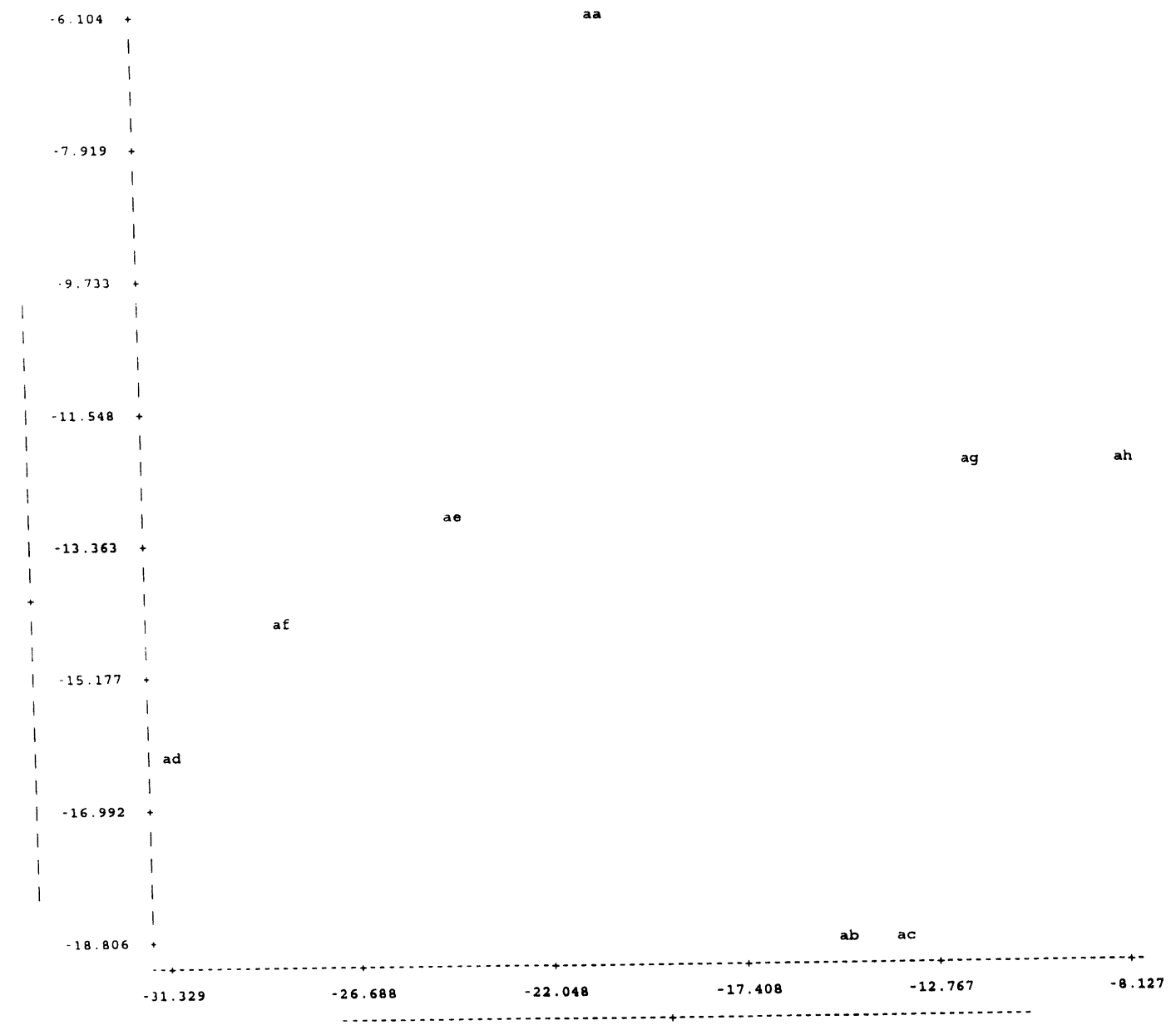


Figure 4. Plot of principal components of fatty acids from isolates of *M. icrococcus kristinae.*

MI-LYLAE X-MI-LYLAE Y-MI-LYLAE

X-axis = Principal Component 1 Y-axis = Principal Component 2

Total samples used: 8

MEAN-X: -4.860 SD-X: 13.527
MEAN-Y: -7.410 SD-Y: 4.875

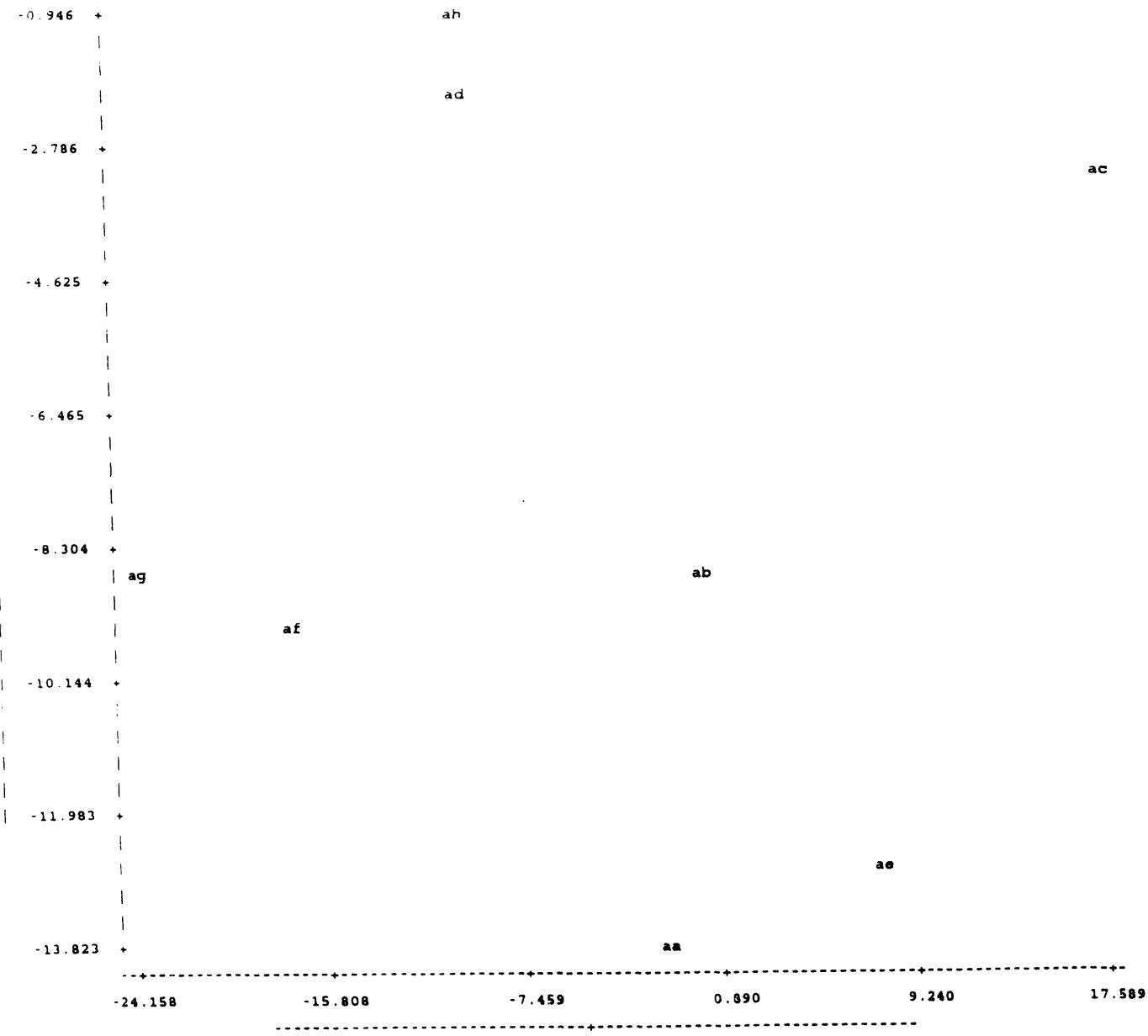


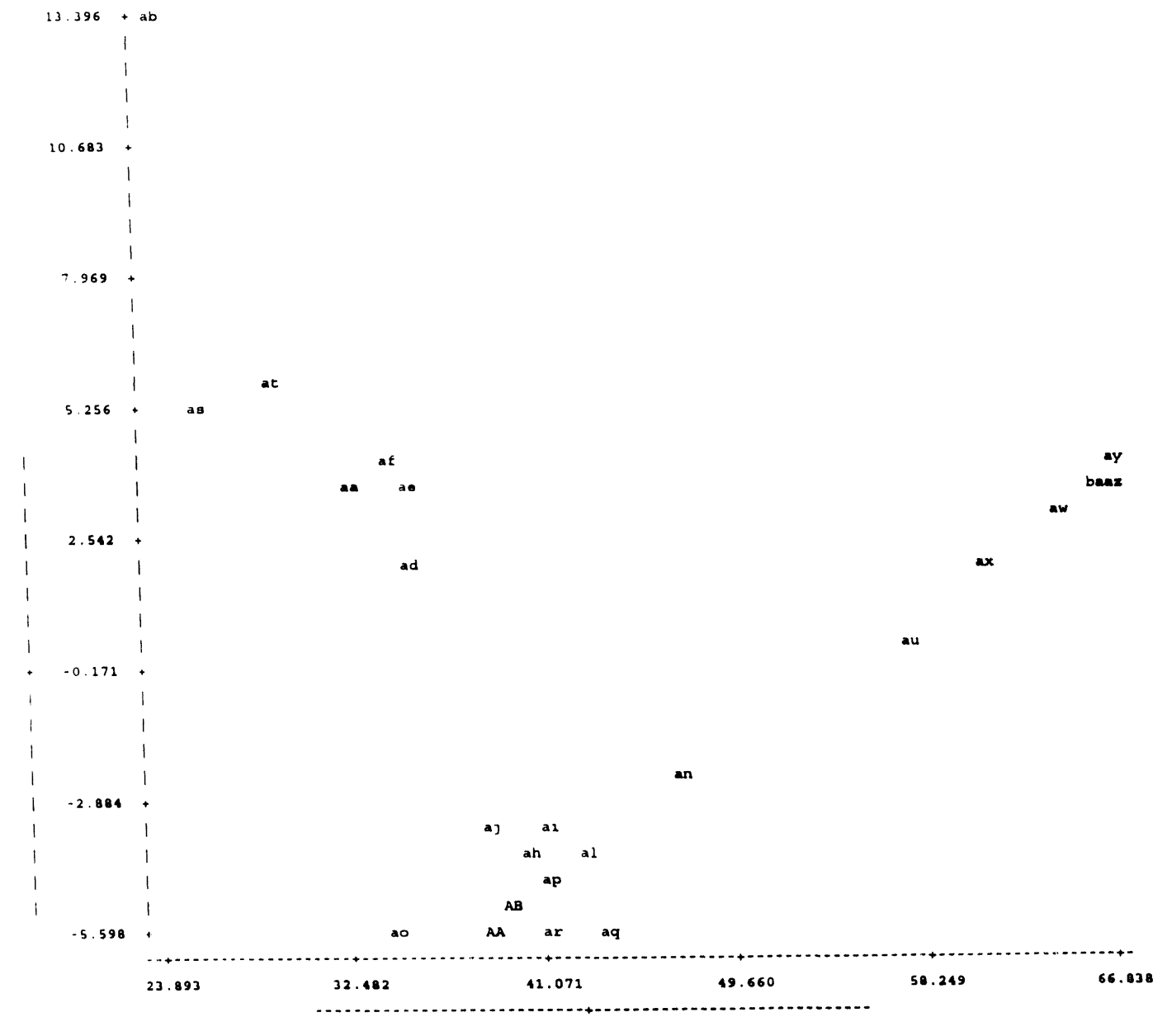
Figure 5. Plot of principal components of fatty acids from isolates of *M. icrococcus lylae*.

MI-LUTEU X-MI-LUTEU Y-MI-LUTEU

X-axis = Principal Component 1 Y-axis = Principal Component 2

Total samples used: 27

MEAN-X: 43.074 SD-X: 12.406
MEAN-Y: 0.384 SD-Y: 4.994



SAMPLE GROUPS:

AA = ak ag
AB = av am ac

Figure 6. Plot of principal components of fatty acids from isolates of *M. icrococcus luteus.*

[MAPPED FEATURES] NUMBER OF SAMPLES: 14

Index	Feature	Count	Mean	Std Dev	Std Dev / Mean	Minimum Value	Minimum Sample	Maximum Value	Maximum Sample	Feature Status
8	11:0 ANTEISO	1	0.34	1.28	3.74	0.00	1	4.78	6	<NOT USED>
24	13:0 ISO	3	1.26	2.68	2.13	0.00	1	8.45	6	
25	13:0 ANTEISO	8	3.74	4.09	1.09	0.00	1	11.65	6	
34	14:0 ISO	12	7.37	4.00	0.54	0.00	3	14.25	6	
37	14:0	14	6.97	2.21	0.32	3.14	2	13.11	6	
43	15:1 ISO G	3	1.23	2.54	2.06	0.00	1	6.92	7	
45	15:1 ANTEISO A	3	1.79	4.06	2.26	0.00	1	11.43	8	
46	15:0 ISO	14	31.05	4.38	0.14	24.41	12	36.22	4	
47	15:0 ANTEISO	14	55.32	8.87	0.16	43.72	2	66.60	12	
49	15:1 w6c	1	0.26	0.98	3.74	0.00	2	3.67	1	<NOT USED>
52	15:0	5	2.63	3.87	1.47	0.00	2	10.01	3	
58	16:1 ISO H	2	0.68	1.76	2.58	0.00	2	5.51	1	<NOT USED>
60	16:0 ISO	14	14.06	5.96	0.42	9.15	3	29.56	2	
65	16:1 w7c	8	3.75	3.97	1.06	0.00	2	11.62	3	
67	16:0	14	10.60	5.01	0.47	4.49	13	17.76	7	
69	15:0 2OH	1	0.35	1.32	3.74	0.00	1	4.94	11	<NOT USED>
71	ISO 17:1 w9c	1	0.55	2.07	3.74	0.00	2	7.74	1	<NOT USED>
75	ANTEISO 17:1 w9c	4	2.71	5.03	1.86	0.00	2	15.03	1	
78	17:0 ISO	7	6.49	6.92	1.07	0.00	6	16.93	2	
79	17:0 ANTEISO	14	17.07	6.56	0.38	7.14	6	25.21	1	
82	17:1 w7c	2	0.59	1.51	2.54	0.00	1	4.33	8	<NOT USED>
86	17:0	1	0.30	1.11	3.74	0.00	2	4.15	1	<NOT USED>
99	18:0	3	1.11	2.23	2.01	0.00	2	6.03	7	<NOT USED>
100	17:0 ISO 3OH	1	0.20	0.73	3.74	0.00	1	2.74	2	<NOT USED>
101	17:0 2OH	1	0.23	0.86	3.74	0.00	1	3.24	2	<NOT USED>
105	19:0 ISO	1	0.28	1.06	3.74	0.00	1	3.98	8	<NOT USED>
121	SUMMED FEATURE 2	1	0.27	1.01	3.74	0.00	2	3.78	1	<NOT USED>
* 1	<Principal Comp 1>		9.69	15.21						
* 2	<Principal Comp 2>		-0.86	8.45						
* 3	<Principal Comp 3>		9.35	5.71						
* 4	<Principal Comp 4>		-12.01	4.23						
* 5	<Principal Comp 5>		5.00	3.24						

TRAINING PARAMETERS:

Quality Threshold	0.25	Std Dev Zero Feat	1.00
Maximum Misses	3	Eigenvalue Adder	1.00
Max Feat Std Dev	4.00	Use Cross Terms	T

Figure 7. Chart of fatty acids from *M. icrococcus luteus* used to characterize a new library entry.

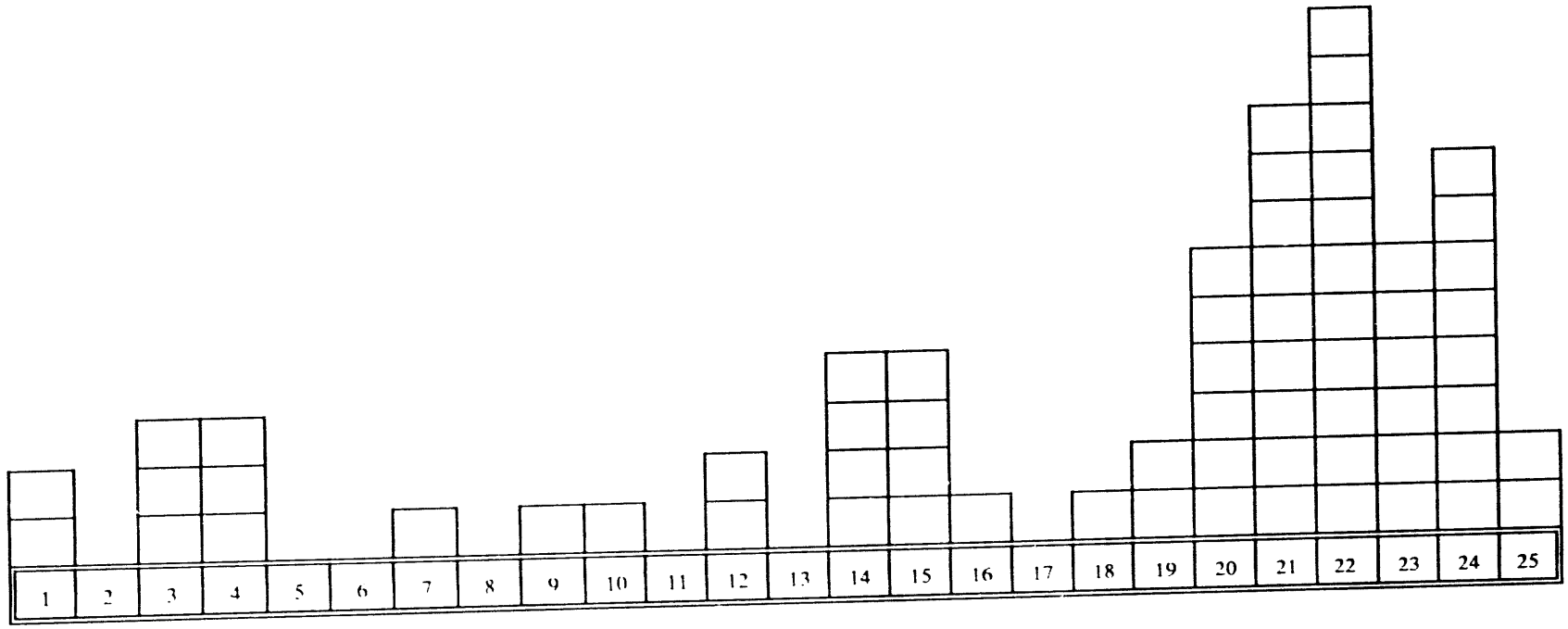


Figure 8. Histogram illustration of *Micrococcus luteus* isolates using principal component # 1.

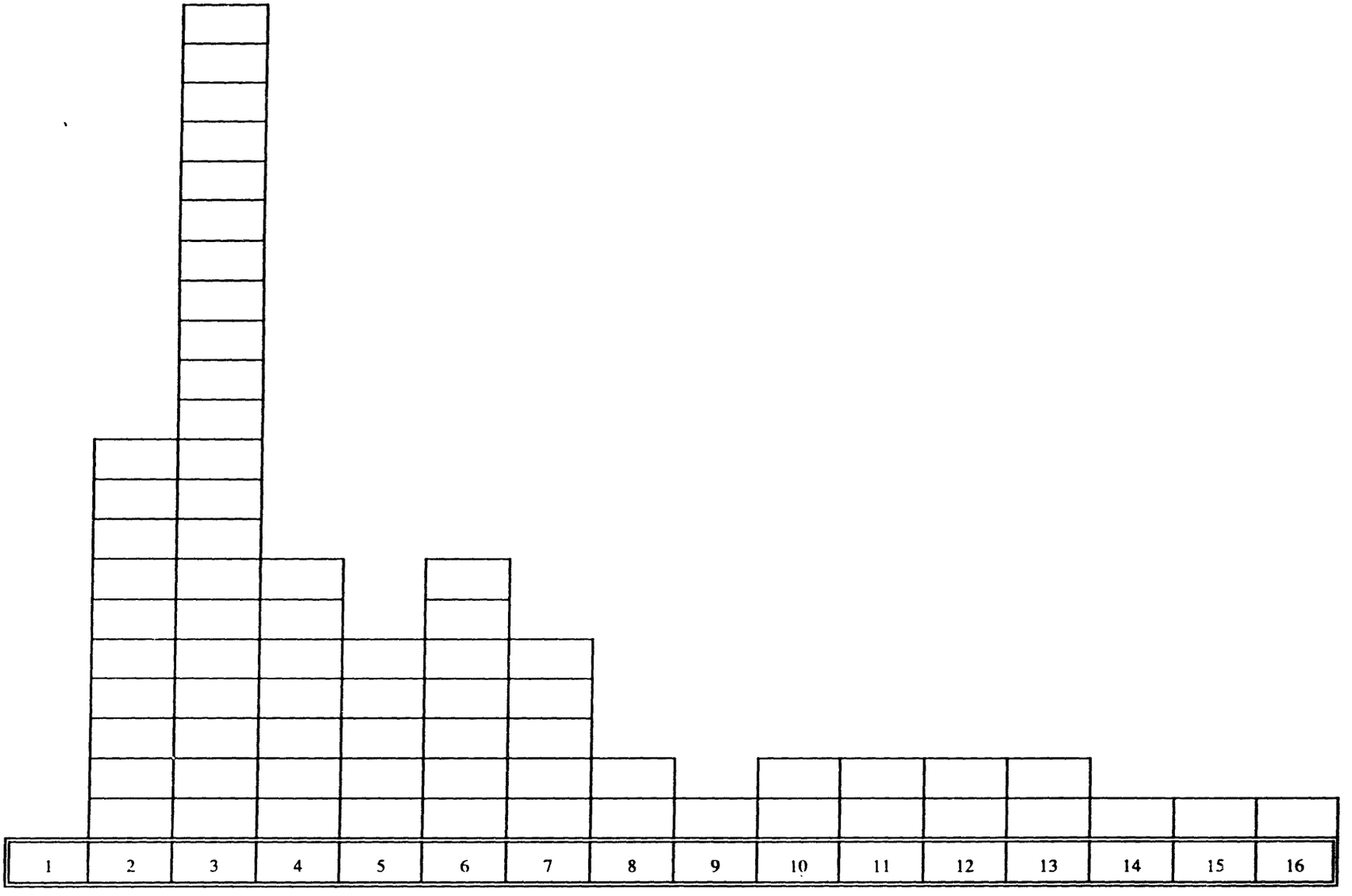


Figure 9. Histogram illustration of *Micrococcus luteus* isolates using distance from the mean.

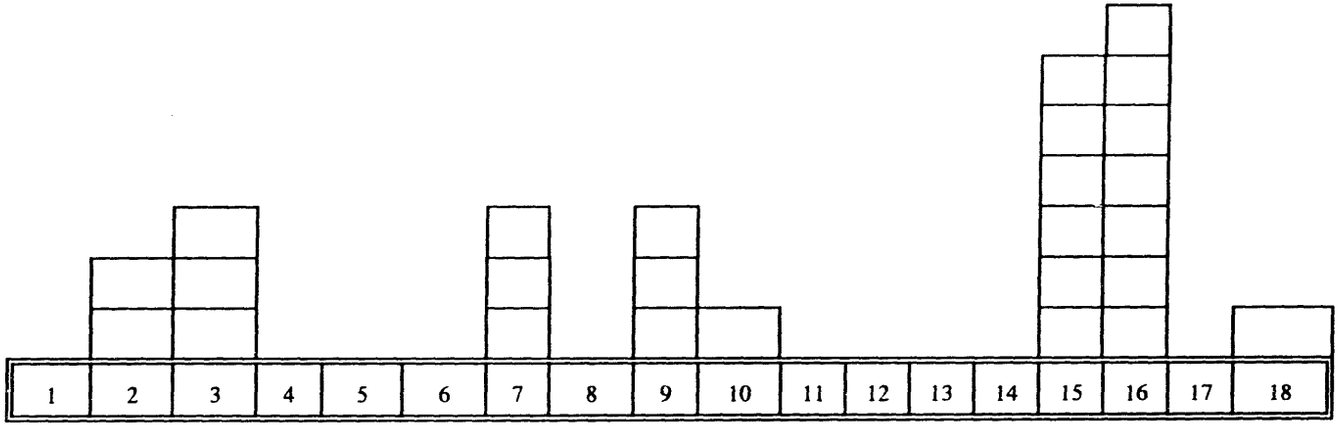


Figure 10. Histogram illustration of taxonomic subgroups using principal components of *Micrococcus luteus* isolates.

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