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Numerical Taxonomy of Anaerobic Bacteria Isolated from Ground Water of a Sanitary Landfill

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NUMERICAL TAXONOMY OF ANAEROBIC BACTERIA ISOLATED FROM GROUND WATER OF A SANITARY LANDFILL

BY

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THESIS

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Preface

The original purpose of this study was to enumerate and characterize the facultative and obligately anaerobic bacteria present in the Orange County sanitary landfill, a landfill characterized by sandy soil and a periodically high water table. The masses of data required in a broad study of this nature made computer analysis and, therefore, numerical taxonomy, the method of choice for this investigation.

The thesis is divided into two sections. The first section describes by numerical taxonomy the organisms isolated from the landfill and identified to genus where possible. The second section is a direct result of the first. In an effort to gain more information from the dendrograms presented in the first section, an index was developed which ranks the organisms within each phenon with respect to their "goodness of fit" in that phenon.

Thus, this thesis provides data concerning both anaerobic bacteria in a sanitary landfill and a unique index which may be of use in numerical taxonomy to aid in the interpretation of dendrograms.

Abstract

Section I

Facultative and obligately anaerobic bacteria were isolated from the ground water of a sanitary landfill characterized by sandy soil and a periodically high water table. Isolates were examined for 63 characteristics and subjected to numerical analysis. Eight clusters were established and correlations with conventional taxonomy were made. The Bacteriodaceae were found to be the dominant group of organisms by the methods employed. The anaerobic population was observed to decrease as the period of seasonal rainfall ended. At the same time, gram positive anaerobes were largely replaced with gram negative ones. Leaching between sampling sites (wells) made correlations between metabolic end products (observed by gas-liquid chromatography) and metabolites produced by the organisms in vitro, impossible. Attempts were made to modify the original test battery to create a smaller battery which would yield approximately the same groupings as the original battery. Clusters became less discreet with these modifications and probably unacceptable for detailed taxonomic work.

Abstract

Section II

An index is described which measures the "goodness of fit" of an organism within a phenon as established by numerical taxonomy. A hypothetical mean organism was established for each phenon. Similarity and relevance coefficients were generated between this hypothetical organism and each member of the phenon. The product of these two coefficients has been termed the Index of Relevance and Similarity (I_{RS}). This index ranges from zero to unity and can be generated with two-state and/or multistate data.

Table of Contents

Section I

The sanitary landfill is the site of both aerobic and anaerobic decomposition of solid waste materials. Characteristic organisms found in these landfills have been described but emphasis has been on aerobic organisms (6, 8, 15).

In Orange County, Florida, a sanitary landfill was opened in 1971, in a high water table area where the soil is primarily sand. The site is underlain with a hardpan layer that impedes downward percolation. This type of geological formation enhances saturation of the buried waste materials with water. Anaerobic decomposition assumes greater importance here than in a more typical landfill condition. Decomposition products of anaerobic metabolism as leachates and associated microorganisms then become a factor in the ecology of the affected ground water.

Two types of cells were constructed in the Orange County landfill area. One series was constructed with dewatering ditches around each cell. The other series of cells consisted of 2.4 m trenches which permit ground water to contact waste material. Shallow wells

(max 9.1 m) were installed within, through, and at varying distances and depths around the fill areas. These wells allow the monitoring of chemical leachates and microorganisms into the ground water. A complete description of the landfill has been previously reported (4).

The bacteria present in the ground water environment surrounding these landfills have not been thoroughly documented. Characterization of these bacteria by the methods of numerical taxonomy represents an objective way to assimilate data from a variety of analyses (e. g., morphology, physiology, biochemistry, etc.). Since it lends itself to automatic processing, it is more efficient than conventional methods. It has been previously used in ecological studies with success (10, 18). Numerical taxonomy was employed in this study in an attempt to detect ecological and taxonomic relationships in this particular landfill environment.

Materials and Methods

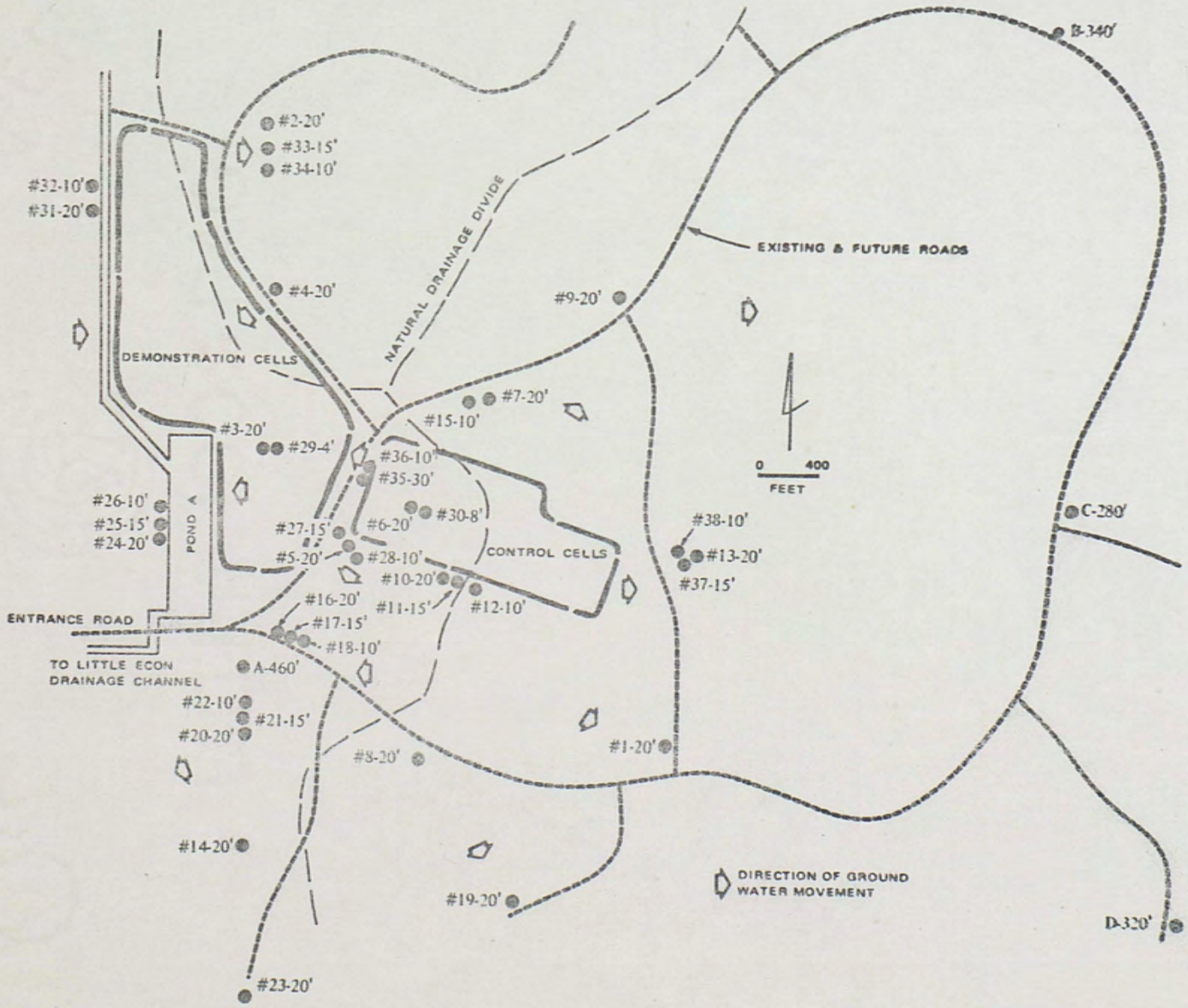
Sampling locations

Wells for monitoring ground water were those used in a previous study (4). Four wells numbered 3, 5, 10, and 29 were choosen for sampling sites based on their location with respect to the cells of buried waste material (Fig. 1). Well 29 is 1.2 m deep extending directly into the solid waste material. Well 3 is 6.1 m deep extending through and beneath the same cell occuppied by well 29. Wells 5 and 10 are also 6.1 m deep and are located lateral to waste cells to monitor horizontal leaching. These four wells allow sampling at successively greater distances from the waste material.

Isolation of strains

Ground water samples were extracted from the wells into clear Erlenmyer flasks through Tygon tubing employing a vacuum pump with adjustable vacuum control. Water flow was regulated to minimize aeration and the tubing was flushed with at least 4 l of water prior to sampling to remove flora from the previous sample. The flasks were filled slowly to capacity and rubber stoppered to minimize air space. Those samples which

Fig. 1. Locations of ground water sampling sites. From "Effective use of high water table areas for sanitary landfill", p. 77, (4).


B-340'
#2-20'
#33-15'
#34-10'
#32-10'
#31-20'
NATURAL DRAINAGE DIVIDE
EXISTING & FUTURE ROADS
#4-20'
#9-20'
DEMONSTRATION CELLS
#7-20'
#15-10'
#3-20'
#29-4'
#36-10'
#35-30'
0 400
FEET
#26-10'
#25-15'
#24-20'
POND A
#30-8'
C-280'
#38-10'
#27-15'
#6-20'
#5-20'
#28-10'
CONTROL CELLS
#13-20'
#37-15'
#10-20'
#16-20'
#11-15'
#12-10'
ENTRANCE ROAD
#17-15'
#18-10'
TO LITTLE ECON
DRAINAGE CHANNEL
A-460'
#22-10'
#21-15'
#20-20'
#8-20'
#1-20'
#14-20'
DIRECTION OF GROUND
WATER MOVEMENT
#19-20'
D-320'
#23-20'

were to be used for anaerobe isolation were collected in flasks which contained 10% (vol/vol) compost extract. Compost consisted of 2 year old grass clippings and other plant matter. This material was extracted by stirring with an equal volume of water for at least 1 h without heat. After stirring, the mixture was clarified by centrifugation at low speed. Sterilization was effected by autoclaving (121 C for 15 min).

The temperature of the ground water was taken in situ with a telethermometer. The pH of each well sample was taken with a pH meter immediately upon return to the laboratory from those samples which did not contain compost extract.

Samples for anaerobic isolation were placed in an anaerobic chamber (Coy Mfg. Co., Ann Arbor) similar to the one described by Aranki et al. (2). Appropriate dilutions were established in peptone-yeast extract-glucose broth (PYG) and standard plates were made on PYG agar. These media are described by Holdeman and Moore (9) but do not contain cysteine or resazurin since preliminary work indicated adequate prereduction in the anaerobic chamber. Water from well 5 was substituted for distilled water.

Spread plates composed of phenylethanol agar (Difco)

in the recommended concentration supplemented with 5% (vol/vol) sheep red blood cells and 1% (wt/vol) vitamin K-hemin solution (9) (herafter referred to as PEA) were employed to isolate gram positive organisms.

Colony forming units (CFU) were counted and isolates were selected after 48-72 h. The spread plates were selected from predetermined portions of the plate to insure random selection. All media were prereduced in the chamber for at least 24 h prior to use. Isolates were maintained in PYG broth and transferred every 7-10 days. The chamber was maintained at 29±2 C throughout the study.

Morphology and physiology

PYG streak plates were examined for the colonial characteristics listed in Table 1 after 48 h. Gram stains were made on 48 h and 21 day PYG cultures outside the chamber using the Kopeloff modification (17). Individual characteristics listed in Table 1 were determined from the 48 h stain. Refractive bodies found in the 21 day stain were considered to be spores. Isolates changing from gram positive to gram negative in 21 days were considered to be gram variable.

Duplicate PYG slants of each isolate were incubated aerobically at 30 C to establish possible aerotolerance.

Flagellation of each isolate was determined by

Table 1. Characteristics of anaerobic isolates from sanitary landfill ground water surveyed for cluster analysis.

Test	Features scored
I Cell morphology	
1. length	>0.5μ, 0.5-1.2μ, 1.3-3.0μ, <3.0μ, or variable (>75% in any one category)
2. width	slender (LxW 3:1), short (LxW 2-3:1), oval (LxW 1:1), or variable (>75% in any one category)
3. shape	rod, coccus, curved, spiral, branching, or pleomorphic (>75% in any one category)
4. gram reaction	negative, positive, or variable
5. motility	flagellated or not flagellated
6. endospores	observed or not observed
7. cell arrangement	single, pairs, clusters, chains (4 or more cells), packets, filaments (<8μ L; LxW 20:1), variable arrangement
II Colony morphology	
8. size	>2mm, 2-5mm, or <5mm
9. elevation	flat, convex, or umbonate
10. edge	entire, undulate, lobate, erose, filamentous

Table 1 -- Continued

Test	Features scored
II Colony morphology (continued)	
11. chromogenesis	nonpigmented (transparent or translucent), white/ off-white, or pigmented
III Oxygen sensitivity	
12. relation to free oxygen	facultative anaerobe or obligate anaerobe
IV Carbohydrate fermentation	
13. arabinose	positive or negative
14. cellobiose	positive or negative
15. galactose	positive or negative
16. glucose	positive or negative
17. inositol	positive or negative
18. lactose	positive or negative
19. pectin	positive or negative
20. sorbitol	positive or negative
21. sucrose	positive or negative
22. xylose	positive or negative
V Antibiotic sensitivity	
23. penicillin G (10 units)	sensitive or resistant
24. tetracycline (30 mcg)	sensitive or resistant

Table 1 -- Continued

Test	Features scored
V Antibiotic sensitivity (continued)	
25. clindamycin (2 mcg)	sensitive or resistant
26. ampicillin (10 mcg)	sensitive or resistant
VI Glucose end products	
27. formic acid	present or absent
28. acetic acid	present or absent
29. propionic acid	present or absent
30. isobutyric acid	present or absent
31. butyric acid	present or absent
32. isovaleric acid	present or absent
33. valeric acid	present or absent
34. isocaproic acid	present or absent
35. caproic acid	present or absent
36. heptanoic acid	present or absent
37. ethanol	present or absent
38. propanol	present or absent
39. butanol	present or absent
40. isopentanol	present or absent
41. pentanol	present or absent
VII Biochemical tests	
42. acetoin	positive or negative

Table 1 -- Continued

Test	Features scored
VII Biochemical tests (continued)	
43. ammonia from peptone	positive or negative
44. catalase	present or absent
45. DNAase	present or absent
46. gelatin	hydrolyzed or not hydrolyzed
47. H_2S from thiosulfate	positive or negative
48. indole	present or absent
49. lecithinase	present or absent
50. lipase	present or absent
51. milk coagulation	positive or negative
52. milk digestion	positive or negative
53. meat proteolysis	positive or negative
54. nitrate reduction	positive or negative
55. RNAase	present or absent
56. starch hydrolysis	hydrolyzed or not hydrolyzed
57. urease	present or absent
VI Threonine conversion	
58. acetic, formic, or lactic acids	present or absent
59. propionic acid	present or absent

Table 1 -- Continued

Test	Features scored
VI Threonine conversion (continued)	
60. butyric acid	present or absent
61. isovaleric acid	present or absent
62. valeric acid	present or absent
63. caproic acid	present or absent

electron microscopy (Hitachi HS-8). A drop of 48 h culture was throughly mixed with a drop of 0.66% (wt/vol) phosphotungstic acid (pH adjusted to 6.8 with 1 N KOH) and removed by capillary action with filter paper. All observations were made at 4000X.

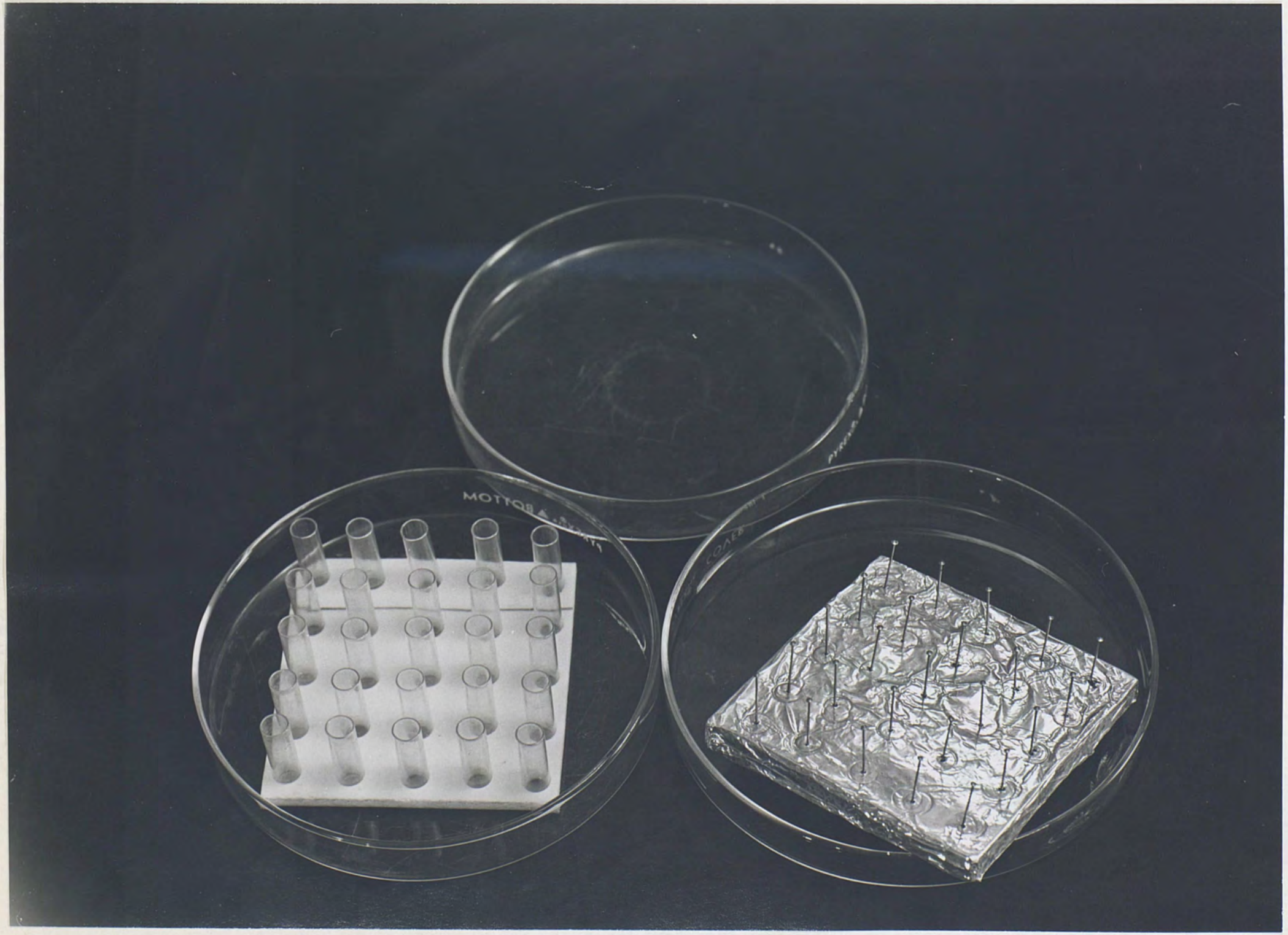
Multipoint analysis

Fermentation of carbohydrates, hydrolysis of starch, presence of urease, and production of H_2S from thiosulfate were studied employing a multipoint inoculating apparatus (Fig. 2). The unit was composed of a 5 x 5 matrix of 6 x 30 mm glass shell vials mounted in autoclavable plastic and maintained in 20 x 150 mm glass petri dishes. The inoculator consisted of a similar matrix of stainless steel straight pins pressed into balsa wood with the head ends down. Both units were sterilized by autoclaving (121 C for 15 min).

A 0.5% (wt/vol) solution of arabinose and a 1% (wt/vol) solution of all other carbohydrates as listed in Table 1 were prepared in PY base (9) employing water from well 5 as solvent. Cysteine and resazurin were ommitted from any of the media described, relying on the reduced atmosphere of the chamber for reduction. PY-urea broth and H_2S medium were prepared according to Holdeman and Moore (9).

Media were dispensed in 1.2 ml aliquots and inocu-

Fig. 2. Multipoint system showing inoculator and shell vial matrix.



lated with cultures not more than 4 days old. Incubation was for 3 days. Fermentation of carbohydrates was detected by addition of the appropriate dye indicator following the incubation period (0.04% [wt/vol] bromcresol green for galactose and xylose fermentation; 0.02% [wt/vol] methyl red for all others). Starch was considered hydrolyzed if no color was apparent upon addition of a few drops of 2% (wt/vol) iodine solution. All multipoint tests were run in duplicate and a blank control was included with each test.

Chromatography

Analysis of carboxylic acid and alcohol metabolites were made from 7 day cultures grown in PYG broth and cultures grown in PY-threonine (9).

A stainless steel column (1.8 m by 32 mm) was packed with 10% SP1200 in 1% H_3PO_4 on 80/100 Chromasorb W AW (Supelco, Inc.). Metabolites from the fermentation of glucose were separated on a Hewlett-Packard 7620A gas-liquid chromatograph with a flame ionization detector. Temperature programming was employed: 90 C for 2 min, increase 15 C/min to 120 C; hold at 120 C for 2 min, increase 30 C/min to 150 C; hold at 150 C for 2 min, increase 30 C/min to 180 C; hold at 180 C for 4 min to end cycle. The carrier gas was nitrogen (20 ml/min) and the injection port was 250 C.

A 5 µl sample of centrifuged PYG broth was routinely injected with a Glenco microsyringe. The fermentation products studied are listed in Table 1.

Response peaks of standards (10 meq/l) of monocarboxylic acids and alcohols which were determined by gas-liquid chromatography (GLC) are in Fig. 3. Retention times varied slightly as determined by the standards.

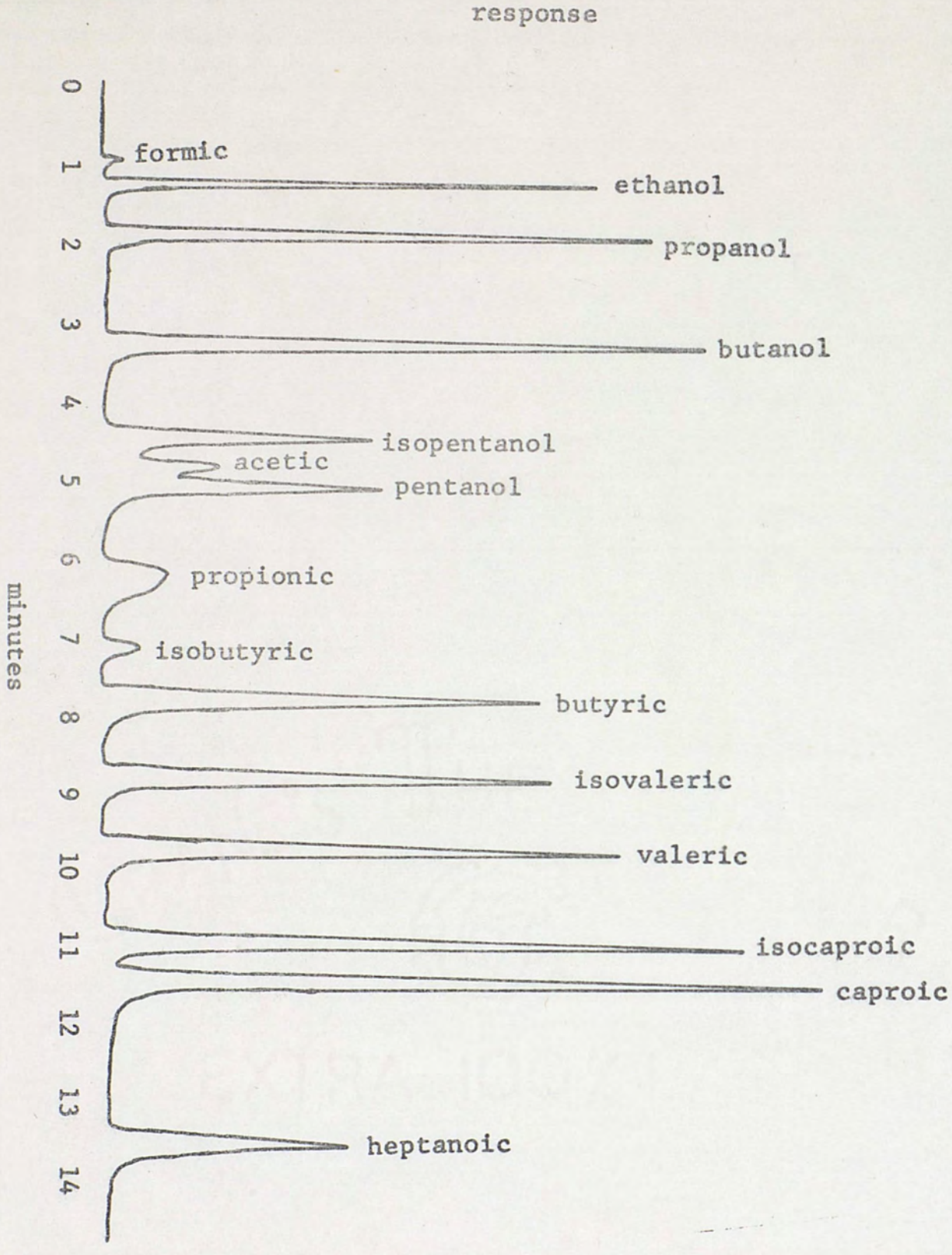
End products of the metabolism of PY-threonine were detected by paper chromatography using a modification of a method by Slifkin and Hercher (21). This method utilizes ethylamine derivatives of carboxylic acids with separation in water saturated butanol. The chromatogram was developed for 14 h, air-dried, and visualized by dipping in 0.2% (wt/vol) bromphenol blue in 95% ethanol.

Miscellaneous biochemical methods

Acetoin and ammonia (from peptone) were determined from 5 day cultures of PYG broth with reagents described by Holdeman and Moore (9).

Three day cultures on McClung-Toabe egg yolk agar (Difco) were examined for lipase and lecithinase activity. After 30 min incubation under aerobic conditions these plates were flooded with 3% H_2O_2. Evolution of gas was indicative of the presence of catalase.

Fig. 3. Gas-liquid chromatogram of standard carboxylic acids and alcohols. Each chemical species was at a concentration of 10 meq/l. A 5 μl aliquot was injected.


response
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
minutes
formic
ethanol
propanol
butanol
isopentanol
acetic
pentanol
propionic
isobutyric
butyric
isovaleric
valeric
isocaproic
caproic
heptanoic

Standard (6 mm diameter) antibiotic sensitivity discs (Difco) containing penicillin G, tetracycline, clindamycin, or ampicillin (Table 1) were dispensed as a set of four on an 18 ml PYG agar plate freshly swabbed with inoculum. Zones of inhibition greater than 1 cm from the center of the disc after 3 days of incubation were considered indicative of sensitivity.

Nitrate reduction and gelatin hydrolysis were performed as a single tube test described by Ball and Sellers (3). Incubation was for 7 days.

Coagulation or digestion of milk was determined after 30 days incubation in PY broth containing 10% (wt/vol) skim milk (Difco). Degradation of meat particles in cooked meat medium (Difco) after 30 days incubation was considered indicative of proteolysis. Indole production was also tested in this medium after 30 days by the addition of Kovács reagent (17).

Presence of ribonuclease (RNAase) or deoxyribonuclease (DNAase) was determined by culturing isolates on a medium containing 2.5% (wt/vol) brain heart infusion, 0.5% (wt/vol) yeast extract, and 1.5% (wt/vol) agar (all Difco) and either 0.25% (wt/vol) RNA (Sigma) or DNA (Difco). After 3 days incubation plates were flooded with 1.5 N HCl. Clear zones around areas of growth were considered as indicative of the presence

of the nucleases (16).

Computer analysis

Similarity coefficients were computed with an IBM 360 model 65 computer using a Fortran IV program modified from Quadling (19) combined with a sort routine by Singleton (20). Jaccard's coefficient was employed (22) which disgards negative matches. Data was coded according to method II of Lockhart (13). Clustering was by the single linkage method (12).

Results

The average number of CFU's per ml of sample is shown in Table 2. Temperature variation was slight throughout the sampling period despite the change of seasons. The pH also showed little fluctuation. Well 29 had significantly higher pH than the other wells. Relative numbers of aerobic CFU's increased with decreasing rainfall while anaerobic CFU's decreased (Tables 2 and 3).

A dendrogram of all organisms isolated is in Fig. 4. Seven distinct phena were designated with an eighth group considered to be a heterogeneous collocation. Reference strains from the literature (5,9) were included to aid in identification. Phena V (Peptostreptococcus) and III (Propionibacterium) represent clusters with the greatest degree of homogeneity. No species of these clusters occur outside their respective phenon. Phenon I (Leuconostoc) was less homogeneous as two species were found to occur outside the cluster. Very few species of the genus Clostridium were isolated with the data showing several Clostridium isolates clustering in phenon VIA. The dendrogram suggests that there is a

Table 2. A comparison of the number of viable colony forming units (CFU) per ml of leachate sample with pH and temperature.

Well	Date	Anaerobic[a] CFU	Aerobic CFU	pH	Temp (C)
3	7/16/74	105000	1700	4.8	23.5
	7/30/74	19000	5200	4.7	24
	12/16/74	6000	15000	[b]	24
	1/11/75	>300	19000	4.6	24
5	7/16/74	34000	340	4.3	24
	7/30/74	730	[b]	4.6	24
	12/16/74	>300	16000	4.5	25
	1/11/75	>300	1500	4.4	24
10	7/16/74	53000	750	6.0	23
	7/30/74	4700	44000	5.0	24
	12/16/74	>300	66000	4.2	24.5
	1/11/75	>300	29000	4.4	23.5
29	7/16/74	90000	110000	5.6	25
	7/30/74	160000	[b]	5.1	28
	12/16/74	44000	18000	6.1	24
	1/11/75	7800	17000	5.8	24

[a]Anaerobic CFU's include both facultative and obligate anaerobes. They have been corrected for the diluting affect of compost extract mixed with the sample. All counts were taken from peptone-yeast extract-glucose agar plates.

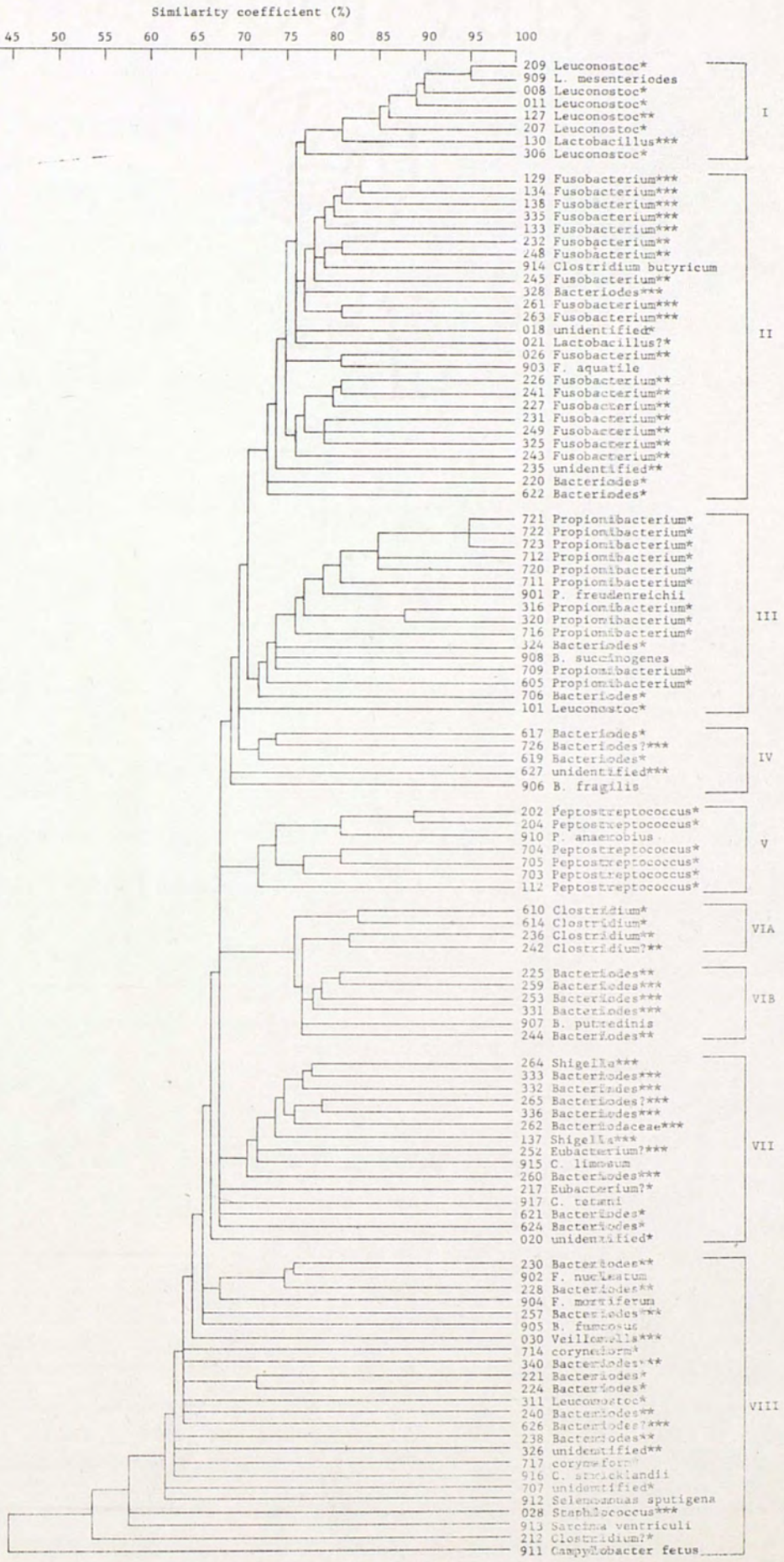
[b]data not available

Table 3. Monthly rainfall from June 1974 to April 1975[a].

Date	Centimeters
June 1974	38.61
July 1974	15.27
August 1974	16.66
September 1974	14.68
October 1974	1.22
November 1974	0.79
December 1974	4.11
January 1975	2.49
February 1975	3.78
March 1975	2.79
April 1975	3.54

[a]Source: National Weather Service

Fig. 4. Dendrogram of isolates from sanitary landfill and selected reference strains after single linkage clustering. Strain numbers correspond to isolation sites: 001-099, well 5; 101-199, well 10; 201-299/601-699, well 29; 301-399/701-799, well 3; 901-999, reference strains. Isolation dates are designated: 7/30/74, (*); 12/16/74, (**); 1/11/75, (***).


Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
209 Leuconostoc*
909 L. mesenteriodes
008 Leuconostoc*
011 Leuconostoc*
127 Leuconostoc**
207 Leuconostoc*
130 Lactobacillus***
306 Leuconostoc*
I
129 Fusobacterium***
134 Fusobacterium***
138 Fusobacterium***
335 Fusobacterium***
133 Fusobacterium***
232 Fusobacterium**
248 Fusobacterium**
914 Clostridium butyricum
245 Fusobacterium**
328 Bacteriodes***
261 Fusobacterium***
263 Fusobacterium***
018 unidentified*
021 Lactobacillus?*
026 Fusobacterium**
903 F. aquatile
226 Fusobacterium**
241 Fusobacterium**
227 Fusobacterium**
231 Fusobacterium**
249 Fusobacterium**
325 Fusobacterium**
243 Fusobacterium**
235 unidentified**
220 Bacteriodes*
622 Bacteriodes*
II
721 Propionibacterium*
722 Propionibacterium*
723 Propionibacterium*
712 Propionibacterium*
720 Propionibacterium*
711 Propionibacterium*
901 P. freudenreichii
316 Propionibacterium*
320 Propionibacterium*
716 Propionibacterium*
324 Bacteriodes*
908 B. succinogenes
709 Propionibacterium*
605 Propionibacterium*
706 Bacteriodes*
101 Leuconostoc*
III
617 Bacteriodes*
726 Bacteriodes?***
619 Bacteriodes*
627 unidentified***
906 B. fragilis
IV
202 Peptostreptococcus*
204 Peptostreptococcus*
910 P. anaerobius
704 Peptostreptococcus*
705 Peptostreptococcus*
703 Peptostreptococcus*
112 Peptostreptococcus*
V
610 Clostridium*
614 Clostridium*
236 Clostridium**
242 Clostridium?**
VIA
225 Bacteriodes**
259 Bacteriodes***
253 Bacteriodes***
331 Bacteriodes***
907 B. puttedinis
244 Bacteriodes**
VIB
264 Shigella***
333 Bacteriodes***
332 Bacteriodes***
265 Bacteriodes?***
336 Bacteriodes***
262 Bacteriodaceae***
137 Shigella***
252 Eubacterium?***
915 C. limosum
260 Bacteriodes***
217 Eubacterium?*
917 C. tetani
621 Bacteriodes*
624 Bacteriodes*
020 unidentified*
VII
230 Bacteriodes**
902 F. nucleatum
228 Bacteriodes**
904 F. mortiferum
257 Bacteriodes***
905 B. fumosus
030 Veillonella***
714 coryneform*
340 Bacteriodes***
221 Bacteriodes*
224 Bacteriodes*
311 Leuconostoc*
240 Bacteriodes**
626 Bacteriodes?***
238 Bacteriodes**
326 unidentified**
717 coryneform*
916 C. sticklandii
707 unidentified*
912 Selenomonas sputigena
028 Staphylococcus***
913 Sarcina ventriculi
212 Clostridium?*
911 Campylobacter fetus
VIII

relationship between certain Clostridium spp. and Bacteriodes spp. in phenon VIB. The clostridia are a highly heterogeneous group and are found scattered through the dendrogram. The reference strains of Clostridium clustered in phena II and VII which represent, in part, species of the Bacteriodaceae and in group VIII which contains a number of Bacteriodes spp. All of the fusobacteria isolated were found to cluster in phenon II. Although other organisms were admitted to phenon II the fusobacteria isolated from the landfill do form a homogeneous group. Three reference strains of Fusobacterium were subjected to cluster analysis and only F. aquatile appears in phenon II. F. nucleatum and F. mortiferum were clustered in group VIII, occurring as a subcluster along with two species of Bacteriodes. This would suggest a heterogeneity within the genus Fusobacterium and only one of the subgroups appears to be present at the landfill. The remaining phena are collocations of Bacteriodes spp.

Group VIII contains genera occurring at a density too low to form separate phena, a number of poorly associated Bacteriodes spp., and the reference strains Selenomonas sputigena, Sarcina ventriculi, and Campylobacter fetus. Indeed, these reference strains should not have associated closely with any of the phena

described.

Isolates taken from each well are clustered in separate dendrograms in Figs. 5-10. Isolates from PYG agar are clustered separately from those isolated on PEA. The number of CFU's observed on PEA were too low for accurate population densities to be estimated. No colonies were observed on any PEA spread plates prepared with samples from wells 5 and 10. In addition to low counts several PEA isolates were found to be gram negative Bacteriodes spp. (Figs. 6 and 10).

Most of the gram positive organisms isolated were obtained during the July sampling when rainfall was heavy and the numbers of anaerobic CFU's was relatively high. When the amount of rainfall and the anaerobic population declined in the same period gram positive organisms were largely replaced by gram negative ones. For example, no Fusobacterium spp. were isolated during the July sampling, but they were found in all wells in the December and January samplings.

Figs. 11-14 are gas-liquid chromatograms of well water samples taken on December 16, 1974. Due to the variations in leaching of materials from different wells no direct correlations can be made between the alcohol and carboxylic acid metabolites produced by the isolates in vitro and those found in ground

Fig. 5. Dendrogram of organisms isolated from sanitary landfill well 3 on peptone-yeast extract-glucose agar. Isolation dates are designated: 7/30/74, (*); 12/16/74, (**); 1/11/75, (***).

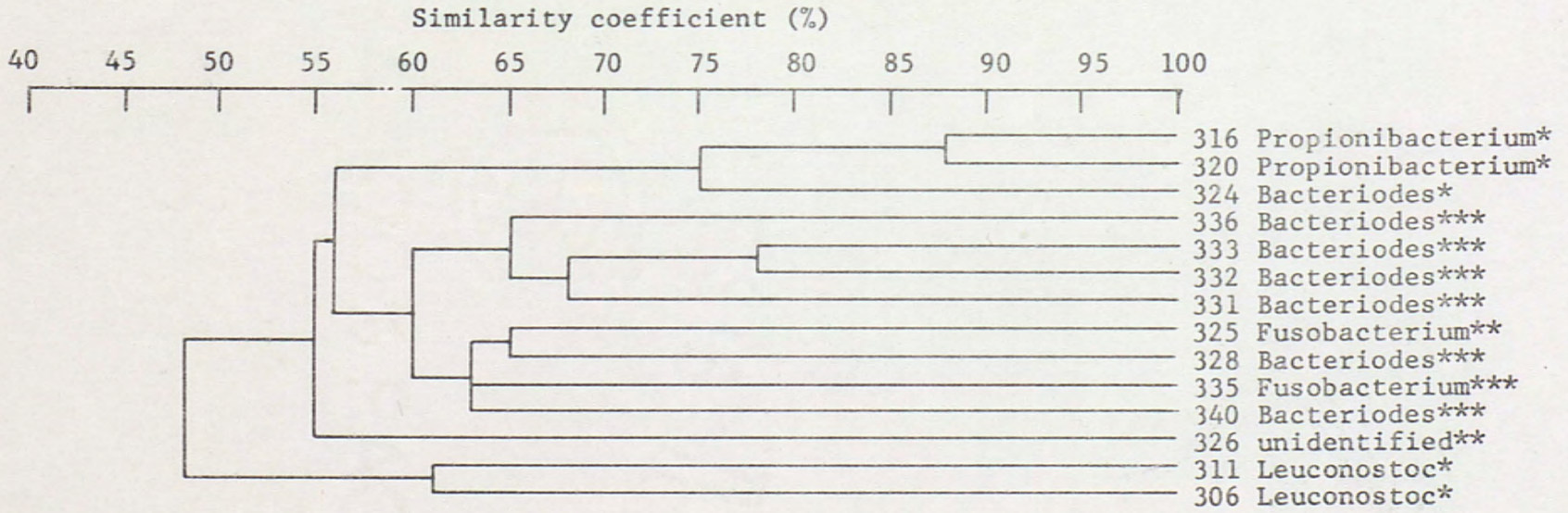

Similarity coefficient (%)
40
45
50
55
60
65
70
75
80
85
90
95
100
316 Propionibacterium*
320 Propionibacterium*
324 Bacteriodes*
336 Bacteriodes***
333 Bacteriodes***
332 Bacteriodes***
331 Bacteriodes***
325 Fusobacterium**
328 Bacteriodes***
335 Fusobacterium***
340 Bacteriodes***
326 unidentified**
311 Leuconostoc*
306 Leuconostoc*

Fig. 6. Dendrogram of organisms isolated from sanitary landfill well 3 on supplemented phenylethanol agar. Isolation dates are designated: 7/30/74, (*); 1/11/75, (***)..

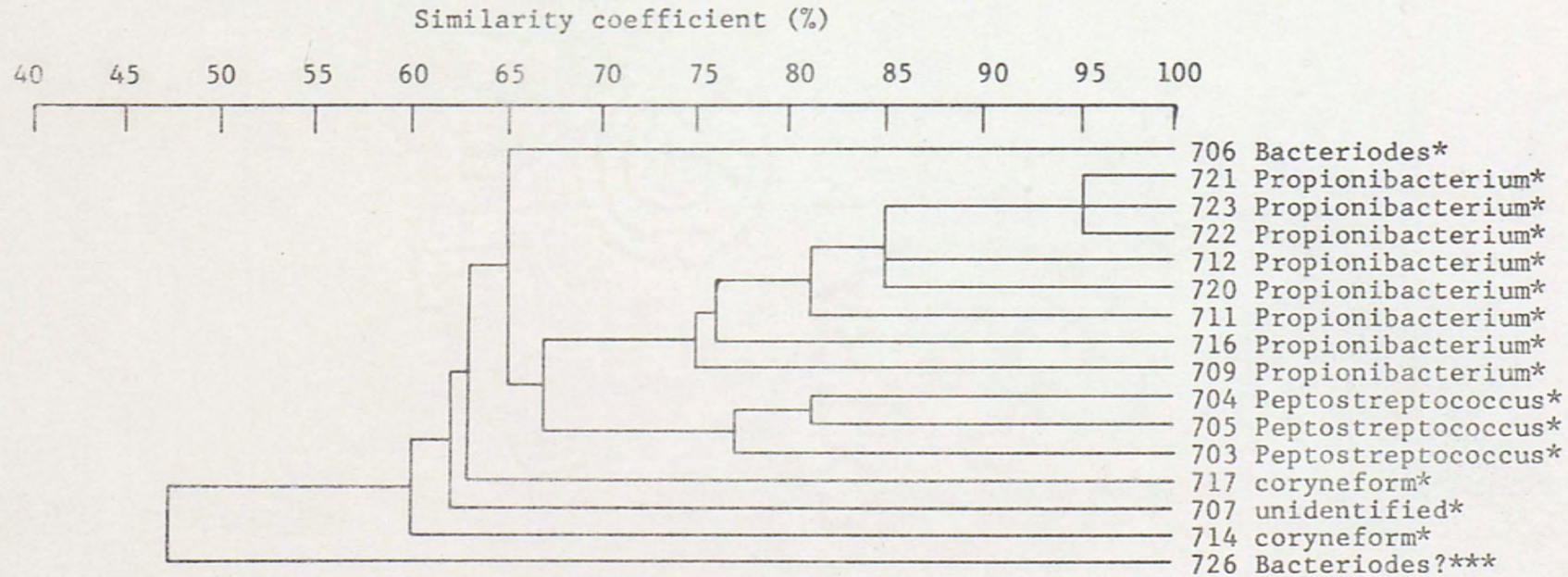

Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
706 Bacteriodes*
721 Propionibacterium*
723 Propionibacterium*
722 Propionibacterium*
712 Propionibacterium*
720 Propionibacterium*
711 Propionibacterium*
716 Propionibacterium*
709 Propionibacterium*
704 Peptostreptococcus*
705 Peptostreptococcus*
703 Peptostreptococcus*
717 coryneform*
707 unidentified*
714 coryneform*
726 Bacteriodes?***

Fig. 7.. Dendrogram of organisms isolated from sanitary landfill well 5 on peptone-yeast extract-glucose agar. Isolation dates are designated: 7/30/74, (*); 12/16/74, (**); 1/11/75, (***).

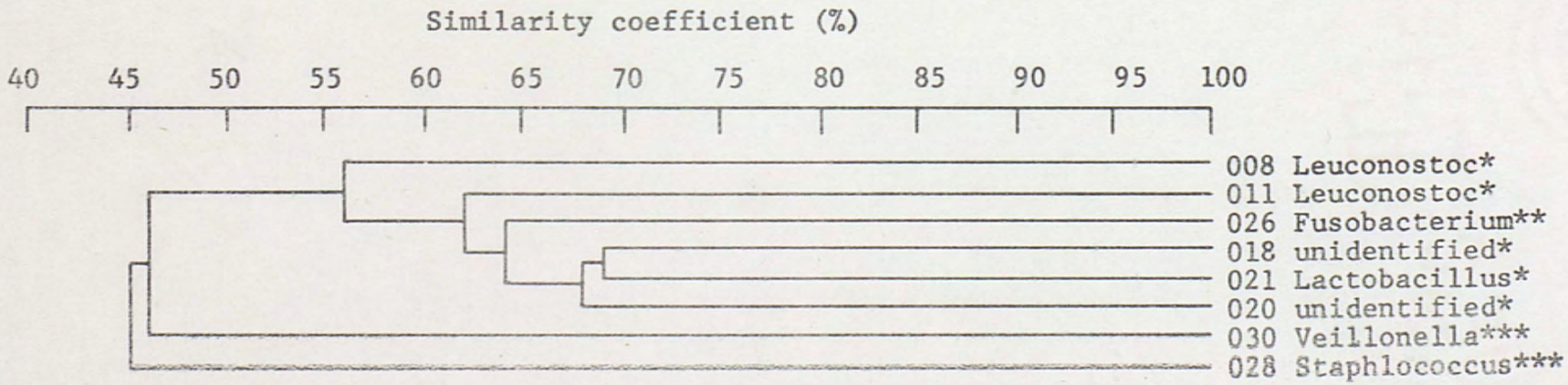

Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
008 Leuconostoc*
011 Leuconostoc*
026 Fusobacterium**
018 unidentified*
021 Lactobacillus*
020 unidentified*
030 Veillonella***
028 Staphlococcus***

Fig. 8. Dendrogram of organisms isolated from sanitary landfill well 10 on peptone-yeast extract-glucose agar. Isolation dates are designated: 7/30/74, (*); 12/16/74, (**); 1/11/75, (***).

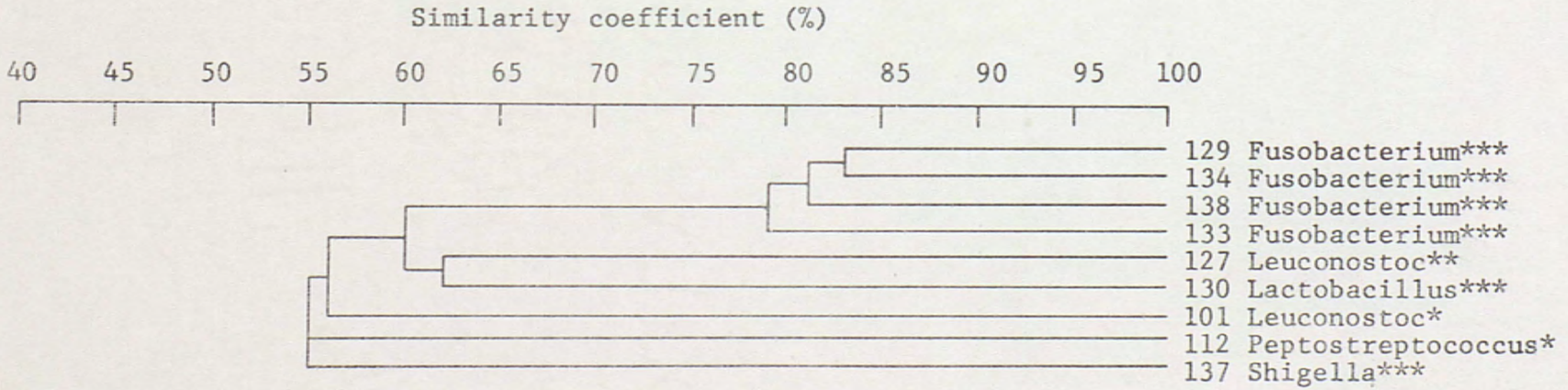

Similarity coefficient (%)
40
45
50
55
60
65
70
75
80
85
90
95
100
129 Fusobacterium***
134 Fusobacterium***
138 Fusobacterium***
133 Fusobacterium***
127 Leuconostoc**
130 Lactobacillus***
101 Leuconostoc*
112 Peptostreptococcus*
137 Shigella***

Fig. 9. Dendrogram of organisms isolated from sanitary landfill well 29 on peptone-yeast extract-glucose agar. Isolation dates are designated: 7/30/74, (*); 12/16/74, (**); 1/11/75, (***).

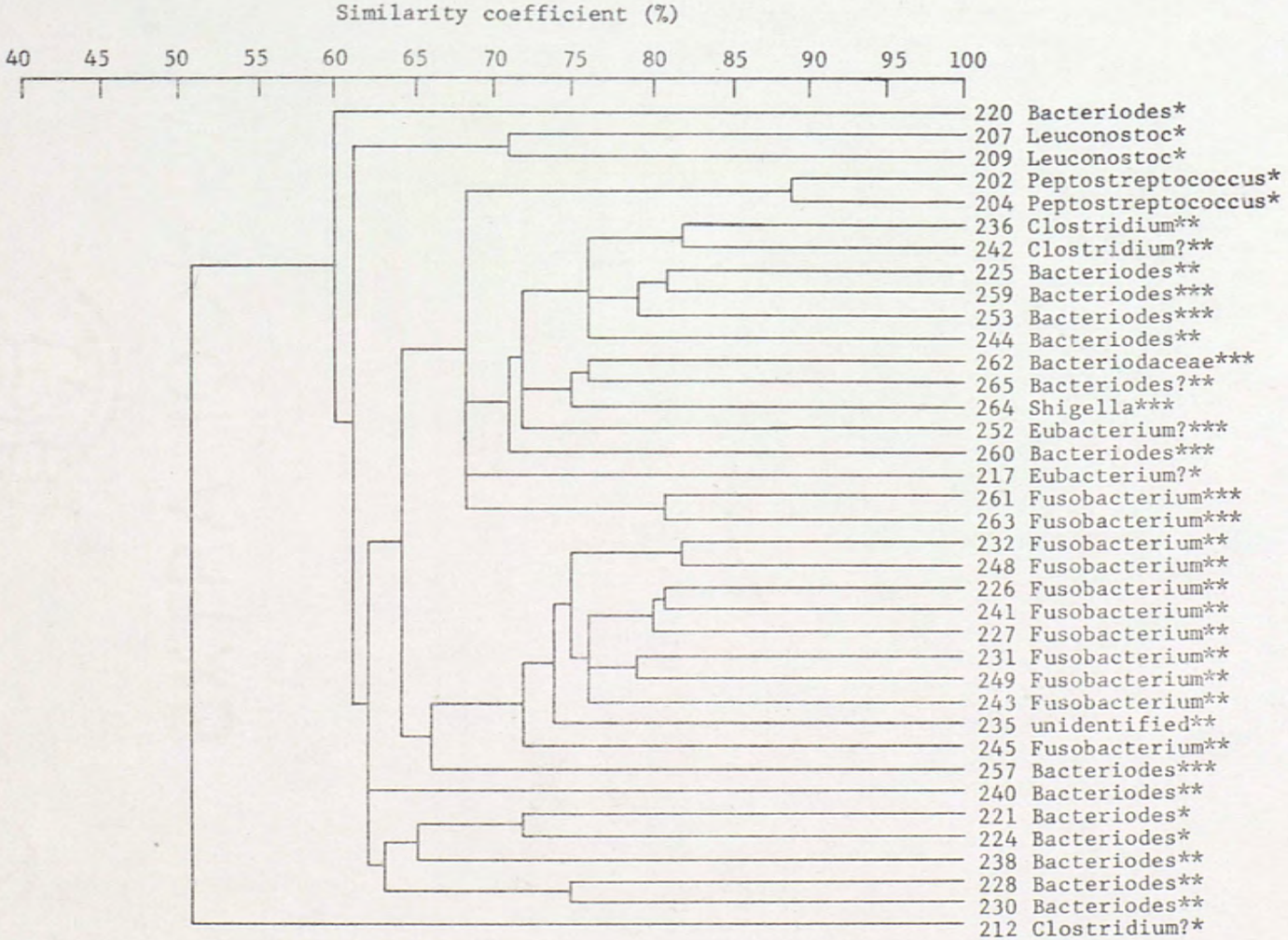

Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
220 Bacteriodes*
207 Leuconostoc*
209 Leuconostoc*
202 Peptostreptococcus*
204 Peptostreptococcus*
236 Clostridium**
242 Clostridium?**
225 Bacteriodes**
259 Bacteriodes***
253 Bacteriodes***
244 Bacteriodes**
262 Bacteriodaceae***
265 Bacteriodes?**
264 Shigella***
252 Eubacterium?***
260 Bacteriodes***
217 Eubacterium?*
261 Fusobacterium***
263 Fusobacterium***
232 Fusobacterium**
248 Fusobacterium**
226 Fusobacterium**
241 Fusobacterium**
227 Fusobacterium**
231 Fusobacterium**
249 Fusobacterium**
243 Fusobacterium**
235 unidentified**
245 Fusobacterium**
257 Bacteriodes***
240 Bacteriodes**
221 Bacteriodes*
224 Bacteriodes*
238 Bacteriodes**
228 Bacteriodes**
230 Bacteriodes**
212 Clostridium?*

Fig. 10. Dendrogram of organisms isolated from sanitary landfill well 29 on phenylethanol agar. Isolation dates are designated: 7/30/74, (*); 1/11/75, (***).

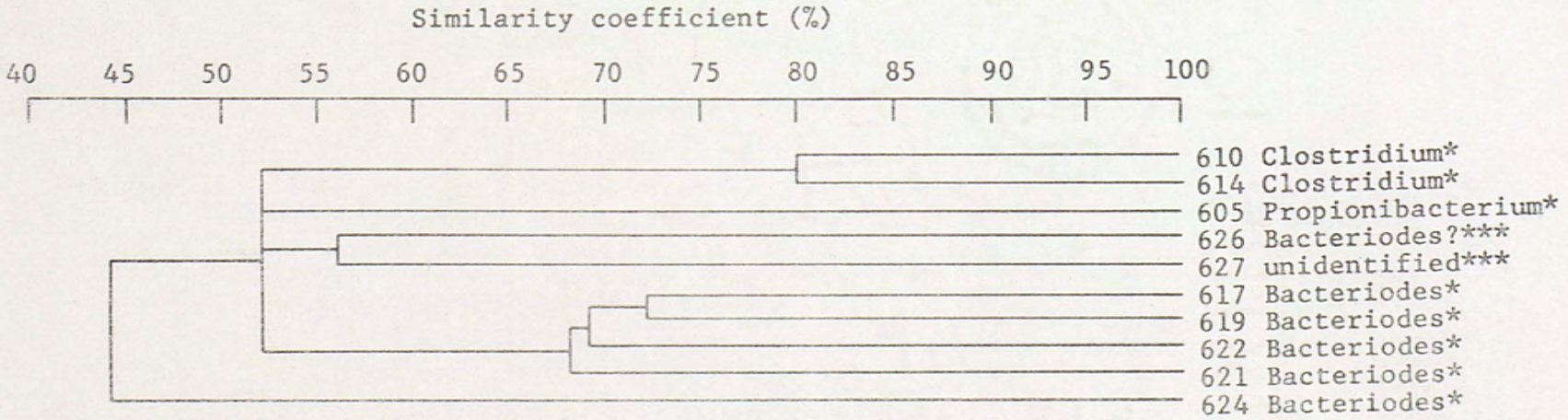

Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
610 Clostridium*
614 Clostridium*
605 Propionibacterium*
626 Bacteriodes?***
627 unidentified***
617 Bacteriodes*
619 Bacteriodes*
622 Bacteriodes*
621 Bacteriodes*
624 Bacteriodes*

Fig. 11. Gas-liquid chromatogram of metabolites in sanitary landfill well 3; 12/16/74.

response
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
minutes
formic
ethanol
propanol
isopentanol
pentanol
isobutyric
butyric
isovaleric
valeric

Fig. 12. Gas-liquid chromatogram of metabolites in sanitary landfill well 5; 12/16/74.

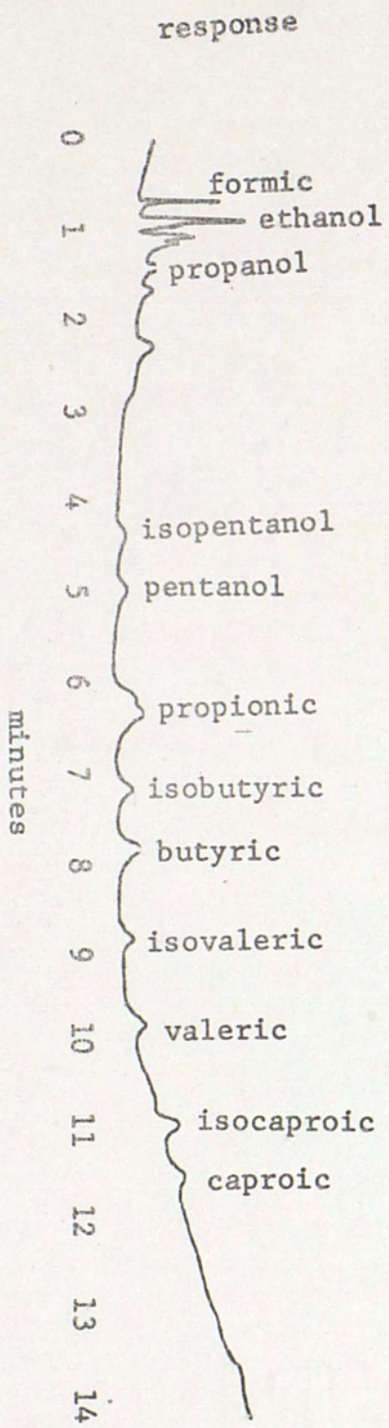

response
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
minutes
formic
ethanol
propanol
isopentanol
pentanol
propionic
isobutyric
butyric
isovaleric
valeric
isocaproic
caproic

Fig. 13. Gas-liquid chromatogram of metabolites in sanitary landfill well 10; 12/16/74.

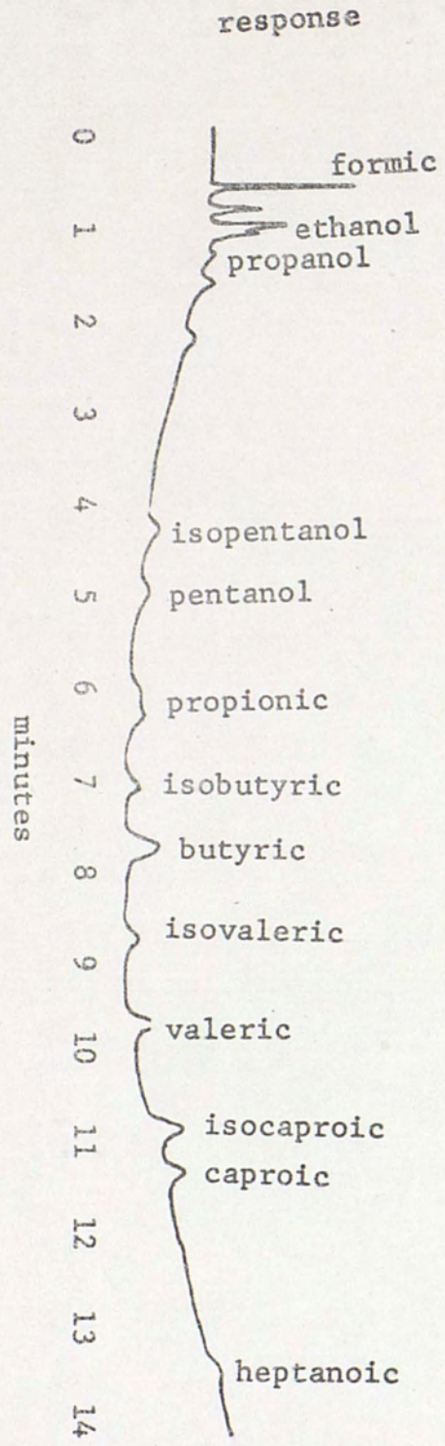

response
formic
ethanol
propanol
isopentanol
pentanol
propionic
isobutyric
butyric
isovaleric
valeric
isocaproic
caproic
heptanoic
minutes
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Fig. 14. Gas-liquid chromatogram of metabolites in sanitary landfill well 29; 12/16/74.

response
formic
ethanol
propanol
isocaproic
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
minutes

water. However, the smallest variety of such metabolites was observed in water taken from well 29.

A smaller test battery would facilitate future studies of this type. Some of the tests employed in this study yielded largely negative results. Since the computer deleted negative matches in the process of establishing similarity coefficients, the influence of these tests on the dendrogram was less than that of the other tests. A modified test battery was instituted which included the 47 tests listed in Table 4. All organisms in the collocation of isolates were again subjected to computer analysis with the resulting dendrogram in Fig. 15. Only phenon V (Peptostreptococcus) maintained its integrity (although there were internal changes) when contrasted with the original dendrogram. Minor changes were observed in phenon III (Propionibacterium). Most of the Fusobacterium spp. in phenon II clustered together but this phenon blends with species of other phena joining the cluster at increasingly lower levels of similarity. The remaining phena are changed considerably from the clusters found in the original dendrogram.

The same reasoning for eliminating individual tests as previously noted was used to develop a battery of 27 tests and all organisms were again subjected to cluster analysis. This battery is in Table 5. Under

Table 4. Abbreviated set of characteristics (47) of anaerobic isolates from sanitary landfill ground water surveyed for cluster analysis in Fig. 15.

Test	Features scored
1. length	>0.5μ, 0.5-1.2μ, 1.3-3.0μ, <3.0μ, or variable (>75% in any one category)
2. width	slender (LxW 3:1), short (LxW 2-3:1), oval (LxW 1:1), or variable (>75% in any one category)
3. shape	rod, coccus, curved, spiral, branching, or pleomorphic (>75% in any one category)
4. gram reaction	negative, positive, or variable
5. motility	flagellated or not flagellated
6. endospores	observed or not observed
7. cell arrangement	single, pairs, clusters, chains (4 or more cells), packets, filaments (<8μ L; LxW 20:1), variable arrangement
8. relation to free oxygen	facultative anaerobe or obligate anaerobe
9. arabinose	positive or negative
10. cellobiose	positive or negative
11. galactose	positive or negative
12. glucose	positive or negative
13. inositol	positive or negative
14. lactose	positive or negative

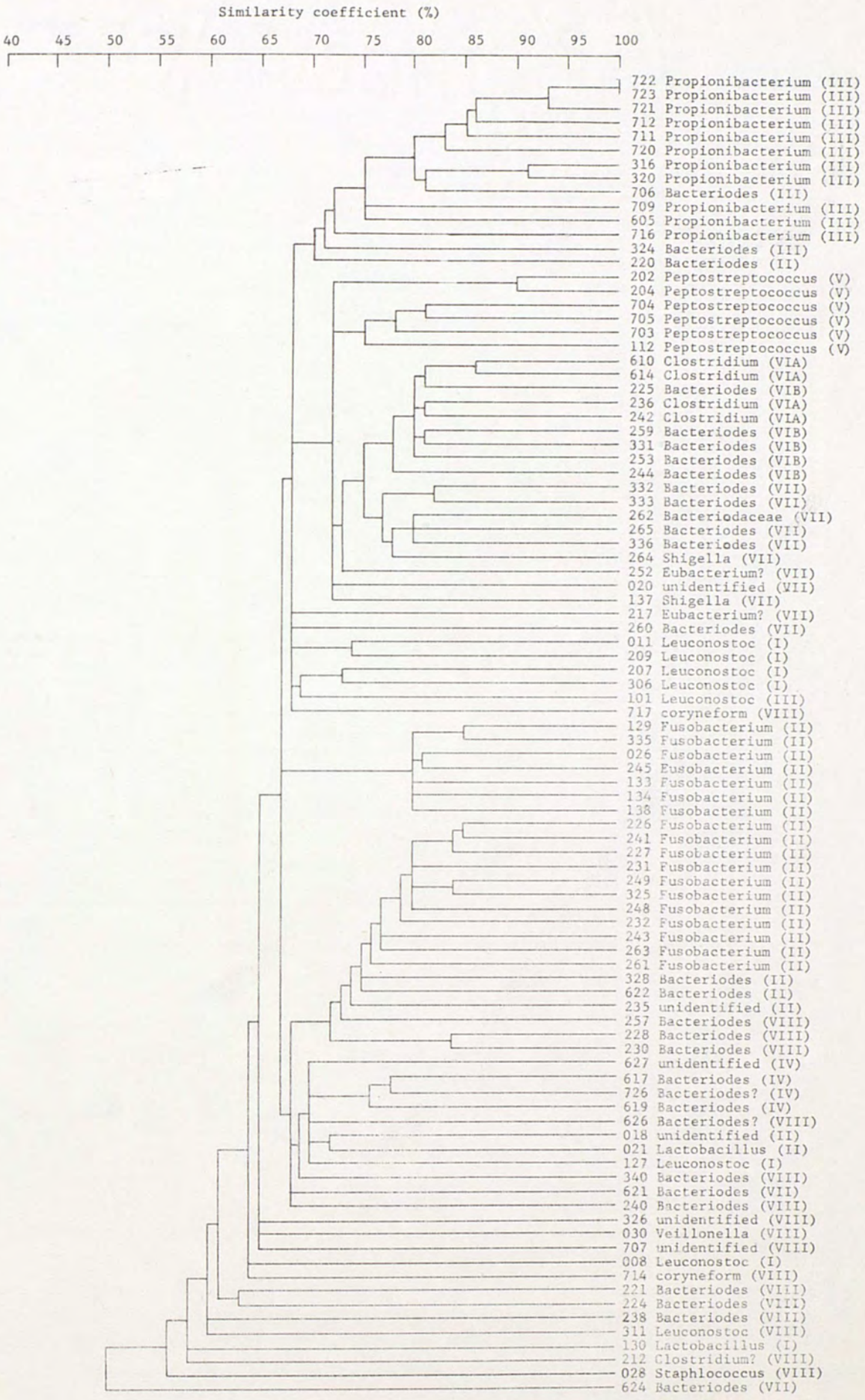
Table 4 -- Continued

Test	Features scored
15. sucrose	positive or negative
16. xylose	positive or negative
17. clindamycin (2 mcg)	sensitive or resistant
18. formic acid	present or absent
19. acetic acid	present or absent
20. propionic acid	present or absent
21. isobutyric acid	present or absent
22. butyric acid	present or absent
23. isovaleric acid	present or absent
24. valeric acid	present or absent
25. isocaproic acid	present or absent
26. caproic acid	present or absent
27. ethanol	present or absent
28. propanol	present or absent
29. butanol	present or absent
30. isopentanol	present or absent
31. pentanol	present or absent
32. ammonia from peptone	positive or negative
33. gelatin	hydrolyzed or not hydrolyzed
34. H_2S from thiosulfate	positive or negative
35. indole	present or absent
36. lecithinase	present or absent

Table 4 -- Continued

Test	Features scored
37. milk coagulation	positive or negative
38. milk digestion	positive or negative
39. meat proteolysis	positive or negative
40. nitrate reduction	positive or negative
41. RNAase	present or absent
42. acetic, formic, or lactic acids from threonine	present or absent
43. propionic acid from threonine	present or absent
44. butyric acid from threonine	present or absent
45. isovaleric acid from threonine	present or absent
46. valeric acid from threonine	present or absent
47. caproic acid from threonine	present or absent

Fig. 15. Dendrogram of isolates based on 47 tests. Roman numerals in parentheses are phenon or group designations from Fig. 4.


Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
722 Propionibacterium (III)
723 Propionibacterium (III)
721 Propionibacterium (III)
712 Propionibacterium (III)
711 Propionibacterium (III)
720 Propionibacterium (III)
316 Propionibacterium (III)
320 Propionibacterium (III)
706 Bacteriodes (III)
709 Propionibacterium (III)
605 Propionibacterium (III)
716 Propionibacterium (III)
324 Bacteriodes (III)
220 Bacteriodes (II)
202 Peptostreptococcus (V)
204 Peptostreptococcus (V)
704 Peptostreptococcus (V)
705 Peptostreptococcus (V)
703 Peptostreptococcus (V)
112 Peptostreptococcus (V)
610 Clostridium (VIA)
614 Clostridium (VIA)
225 Bacteriodes (VIB)
236 Clostridium (VIA)
242 Clostridium (VIA)
259 Bacteriodes (VIB)
331 Bacteriodes (VIB)
253 Bacteriodes (VIB)
244 Bacteriodes (VIB)
332 Bacteriodes (VII)
333 Bacteriodes (VII)
262 Bacteriodaceae (VII)
265 Bacteriodes (VII)
336 Bacteriodes (VII)
264 Shigella (VII)
252 Eubacterium? (VII)
020 unidentified (VII)
137 Shigella (VII)
217 Eubacterium? (VII)
260 Bacteriodes (VII)
011 Leuconostoc (I)
209 Leuconostoc (I)
207 Leuconostoc (I)
306 Leuconostoc (I)
101 Leuconostoc (III)
717 coryneform (VIII)
129 Fusobacterium (II)
335 Fusobacterium (II)
026 Fusobacterium (II)
245 Fusobacterium (II)
133 Fusobacterium (II)
134 Fusobacterium (II)
138 Fusobacterium (II)
226 Fusobacterium (II)
241 Fusobacterium (II)
227 Fusobacterium (II)
231 Fusobacterium (II)
249 Fusobacterium (II)
325 Fusobacterium (II)
248 Fusobacterium (II)
232 Fusobacterium (II)
243 Fusobacterium (II)
263 Fusobacterium (II)
261 Fusobacterium (II)
328 Bacteriodes (II)
622 Bacteriodes (II)
235 unidentified (II)
257 Bacteriodes (VIII)
228 Bacteriodes (VIII)
230 Bacteriodes (VIII)
627 unidentified (IV)
617 Bacteriodes (IV)
726 Bacteriodes? (IV)
619 Bacteriodes (IV)
626 Bacteriodes? (VIII)
018 unidentified (II)
021 Lactobacillus (II)
127 Leuconostoc (I)
340 Bacteriodes (VIII)
621 Bacteriodes (VII)
240 Bacteriodes (VIII)
326 unidentified (VIII)
030 Veillonella (VIII)
707 unidentified (VIII)
008 Leuconostoc (I)
714 coryneform (VIII)
221 Bacteriodes (VIII)
224 Bacteriodes (VIII)
238 Bacteriodes (VIII)
311 Leuconostoc (VIII)
130 Lactobacillus (I)
212 Clostridium? (VIII)
028 Staphlococcus (VIII)
624 Bacteriodes (VII)

these abbreviated conditions organisms generally clustered at higher similarity levels but with a loss of definition between phena (Fig. 16).

Table 5. Abbreviated set of characteristics (27) of anaerobic isolates from sanitary landfill ground water surveyed for cluster analysis in Fig. 16.

Test	Features scored
1. length	>0.5μ, 0.5-1.2μ, 1.3-3.0μ, <3.0μ, or variable (>75% in any one category)
2. width	slender (LxW 3:1), short (LxW 2-3:1), oval (LxW 1:1), or variable (>75% in any one category)
3. shape	rod, coccus, curved, spiral, branching, or pleomorphic (>75% in any one category)
4. gram reaction	negative, positive, or variable
5. motility	flagellated or not flagellated
6. endospores	observed or not observed
7. cell arrangement	single, pairs, clusters, chains (4 or more cells), packets, filaments (<8μ L; LxW 20:1), variable arrangement
8. relation to free oxygen	facultative anaerobe or obligate anaerobe
9. arabinose	positive or negative
10. cellobiose	positive or negative
11. glucose	positive or negative
12. sucrose	positive or negative
13. xylose	positive or negative
14. formic acid	present or absent

Table 5 -- Continued

Test	Features scored
15. acetic acid	present or absent
16. propionic acid	present or absent
17. isobutyric acid	present or absent
18. butyric acid	present or absent
19. isovaleric acid	present or absent
20. valeric acid	present or absent
21. caproic acid	present or absent
22. isocaproic acid	present or absent
23. ethanol	present or absent
24. propanol	present or absent
25. butanol	present or absent
26. isopentanol	present or absent
27. pentanol	present or absent

Fig. 16. Dendrogram of isolates based on 27 tests. Roman numerals in parentheses are phenon or group designations. from Fig. 4.


Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
617 Bacteriodes (IV)
726 Bacteriodes? (IV)
622 Bacteriodes (II)
226 Fusobacterium (II)
227 Fusobacterium (II)
231 Fusobacterium (II)
248 Fusobacterium (II)
245 Fusobacterium (II)
241 Fusobacterium (II)
249 Fusobacterium (II)
325 Fusobacterium (II)
138 Fusobacterium (II)
263 Fusobacterium (II)
134 Fusobacterium (II)
328 Bacteriodes (II)
335 Fusobacterium (II)
257 Bacteriodes (VIII)
626 Bacteriodes? (VIII)
133 Fusobacterium (II)
026 Fusobacterium (II)
129 Fusobacterium (II)
261 Fusobacterium (II)
332 Bacteriodes (VII)
333 Bacteriodes (VII)
627 unidentified (IV)
232 Fusobacterium (II)
243 Fusobacterium (II)
264 Shigella (VII)
265 Bacteriodes? (VII)
244 Bacteriodes (VIB)
262 Bacteriodaceae (VII)
340 Bacteriodes (VIII)
137 Shigella (VII)
235 unidentified (II)
260 Bacteriodes (VII)
619 Bacteriodes (IV)
240 Bacteriodes (VIII)
021 Lactobacillus (II)
228 Bacteriodes (VIII)
230 Bacteriodes (VIII)
331 Bacteriodes (VIB)
225 Bacteriodes (VIB)
253 Bacteriodes (VIB)
259 Bacteriodes (VIB)
610 Clostridium (VIA)
614 Clostridium (VIA)
236 Clostridium (VIA)
252 Eubacterium? (VII)
326 unidentified (VIII)
018 unidentified (II)
020 unidentified (VII)
202 Peptostreptococcus (V)
204 Peptostreptococcus (V)
704 Peptostreptococcus (V)
703 Peptostreptococcus (V)
705 Peptostreptococcus (V)
011 Leuconostoc (I)
112 Peptostreptococcus (V)
217 Eubacterium? (VII)
722 Propionibacterium (III)
723 Propionibacterium (III)
721 Propionibacterium (III)
711 Propionibacterium (III)
712 Propionibacterium (III)
720 Propionibacterium (III)
709 Propionibacterium (III)
605 Propionibacterium (III)
316 Propionibacterium (III)
320 Propionibacterium (III)
324 Bacteriodes (III)
706 Bacteriodes (III)
707 unidentified (VIII)
220 Bacteriodes (II)
242 Clostridium? (VIA)
336 Bacteriodes (VII)
030 Veillonella (VIII)
207 Leuconostoc (I)
306 Leuconostoc (I)
209 Leuconostoc (I)
101 Leuconostoc (I)
311 Leuconostoc (VIII)
127 Leuconostoc (I)
224 Bacteriodes (VIII)
714 coryneform (VIII)
130 Lactobacillus (I)
008 Leuconostoc (I)
716 Propionibacterium (III)
238 Bacteriodes (VIII)
028 Staphlococcus (VIII)
621 Bacteriodes (VII)
212 Clostridium? (VIII)
624 Bacteriodes (VII)
221 Bacteriodes (VIII)
717 coryneform (VIII)

Discussion

The data collected show that rainfall is one of the most important variables governing the total number of organisms in the groundwater at the sanitary landfill at a particular time. Previous observations reviewed by Alexander (1) indicate that as soil becomes more saturated it also becomes more anaerobic. It is reasonable to assume that a heavy rainfall period will saturate the waste material, especially in view of the normally high water table, causing the area above the hardpan to become more anaerobic. With the exception of well 29, the depth of which is likely to be above the water table, the number of anaerobic organisms is greater than the number of aerobic organisms when the rainfall is heavy (late Spring to early Fall) and the situation reverses during the periods of slight rainfall.

Well 29 is situated directly in the waste material and therefore the water there contains a greater concentration of degradable substrate than noted in other wells. This well contained the highest total number of organisms at each sampling time. The large number of anaerobes are coincident with large quantities of or-

ganic acids in the water, yet the pH of well 29 water is higher than the other wells. Gas-liquid chromatography of a water sample indicated that there was less acid in 29 than in the other wells. Considering the depth of well 29 metabolites must leach away at a rapid rate under the pressure of heavy rainfall percolation. The loss of these metabolic waste products (and the resultant increase in pH) may, in turn, permit a higher number of organisms to be present.

All of the obligate anaerobes isolated in the study are what Loesche (14) refers to as moderate anaerobes. They can all tolerate a small percentage of oxygen in the atmosphere. If strict anaerobic organisms exist in the landfill they were not recovered with the sampling procedure employed.

Burchinal (6) found Clostridium to be the dominant anaerobic organism in a study of a simulated landfill. In this study Clostridium was found in low numbers which reflect differences in the environment and/or sampling and isolation procedures. The dominant anaerobic organisms recovered from the wells were Bacteriodes spp. Since high numbers of Fusobacterium were also isolated, it would perhaps be more discreet to consider the Bacteriodaceae the dominant group in the landfill (by the methods and conditions employed).

This group formed several phena, each of which differed in the combination of metabolites produced from glucose. It is evident from chromatographic analyses (Figs. 11-14) that a variety of metabolites were produced in the wells. Direct correlation between organisms isolated from each well with metabolites detected there is not appropriate since metabolites can apparently leach into and away from these wells in addition to being produced there. For example, one would expect to find butyric acid in well 29 from the species of Fusobacterium isolated at the same time GLC determination of metabolites present was made (assuming normal carbohydrate fermentation was occurring) (Figs. 9 and 14). If butyric acid was being produced in detectable quantities it must have been leaching away rapidly. The heavy rainfall at this time would account for this.

The low pH of the ground water at the landfill under study precludes the possibility of substantial methane production there at this time (11). Some of the organic acids observed can serve as substrates for methanogenesis (11) and, in time, natural succession of microorganisms in this landfill may lead to methane production on a scale which would make collection of the gas for commercial use feasible. Indeed, this is being done at a landfill elsewhere (7). The possibility of ground water pollu-

tion from materials leaching from the landfill and the potential health problem which may be posed by the high numbers of Bacteriodes spp. found in this study also present themselves. The paucity of literature regarding the anaerobic flora of sanitary landfills indicates a need for more detailed studies in this area of microbial ecology including a long term successional study of the microbial flora found there.

There is no way to estimate the minimum number of tests needed for cluster analysis (22). The 63 tests employed yielded phena with adequate definition for this study. The abbreviated test battery that was suggested (Table 4) should be adequate for reevaluation of the stability of the flora in the ground water of the Orange County landfill. The smaller test battery applied to this diversity of organisms would be appropriate only for use with sequential keys.

Section II

One of the difficulties associated with numerical taxonomy is the interpretation of phena established in a given phenogram. Sneath and Sokal (8) have reviewed various methods for obtaining additional taxonomic information from phenograms. One of these describes a method referred to as the "Peculiarity Index" (1). This index ranks the members of a phenon so that those members which do not fit well in a given phenon may be determined.

Unfortunately the index, as described, can only be applied to two-state data and is rather difficult to calculate. In addition, the index assumes different upper limits for each phenogram which is undesireable (8).

This paper describes an index which ranks the members of phena as above but may be used with two-state or multistate data, ranges from zero to unity, and could be adapted for computerized calculation. The index allows the assessment of the members of phena by statistical analysis and should prove useful in numerical taxonomy.

Materials and Methods

Source and identification of isolates

The organisms described here are facultative and obligate anaerobic bacteria isolated from a sanitary landfill. These isolates were subjected to a battery of 63 tests for the purpose of computer analysis and identifies to genus with a dichotomous key (7). The sampling site, sampling methods, and test battery have been described in section I.

Statistics and computer analysis

All computer analyses were effected with an IBM 360 model 65 computer.

Similarity coefficients were computed using a Fortran IV program modified from Quadling (4) combined with a sort routine by Singleton (6). Negative matches were discarded by Jaccard's coefficient (8). Data were coded according to method II of Lockhart (3). Clustering was accomplished by the single linkage method (2). Phena were established at the discretion of the investigator.

Composite organisms for each cluster were calculated by hand. A characteristic was scored as positive

or negative if at least 2/3 of the organisms in the cluster were respectively scored thus. Multistate characteristics were scored for that state which was in 2/3 majority. If no state was found at this level for a given character it was scored as no comparison.

Similarity coefficients and relevance values were computed between composite organisms and members of their respective clusters using the aforementioned program. Relevance values reflect the percent of the total possible tests (63) which actually were used to compute the similarity coefficients for each pair of organisms. They do not include negative matches between organisms or tests scored for no comparison.

The Index of Relevance and Similarity (I_{RS}) is defined as the product of the relevance value and the similarity coefficient between each member of a cluster and that cluster's composite organism (as described above). I_{RS} values were calculated on a desk calculator.

The basic statistics, viz. mean, Kolmogorov-Smirnov statistic, and associated standard errors and confidence limits were computed with program A3.1 of Sokal and Rohlf (9).

Results

The third and fourth central moments and the Kolmogorov-Smirnov statistic determined for each phenon show that each distribution of I_{RS} values forms a normal distribution (Table 1).

Organisms greater than one standard deviation to the right of the mean (Table 1) were arbitrarily designated as poor members of their respective phena. The critical I_{RS} values used for this determination are in Table 2.

The phena and the individual I_{RS} rankings are given in Fig. 1. Phenon I complies best with classical taxonomy (although that is not a goal of establishing I_{RS}). All the poor members of the phenon are Bacteriodes spp. while the remainder are exclusively Propionibacterium. Phena II, IIIA, and V are each composed of one genus, and each contain one member which fits poorly. The remaining phena show members of the genera of which they are largely composed to be poor members of those respective phena. In some cases minority genera are found to be good members of these phena.

Phenon VI, although largely Bacteriodes, is composed

of members which are grouped at similarity levels relatively lower than those of the rest to the phenogram. This is reflected in the lower I_{RS} values.

Table 1. Basic statistics for I_{RS} values in each cluster in the phenogram (Fig. 1). G1 is the third central moment, G2 is the fourth central moment, and K-S Dmax is the Kolmogorov-Smirnov statistic. (G1, G2, and K-S Dmax show that the distributions are normal.)

Phenon		Statistic	Stand. Error	95% Confid. Limits
I	Mean	0.40500	0.01329	± 0.02844
	Stand. Dev.	0.04973		
	G1	-0.36558	0.59738	± 1.17110
	G2	-1.35741	1.15405	± 2.26239
	K-S Dmax	0.13437		
II	Mean	0.42000	0.02633	± 0.06451
	Stand. Dev.	0.06450		
	G1	-0.46289	0.84515	± 1.65684
	G2	-1.74394	1.74078	± 3.41261
	K-S Dmax	0.15722		
IIIA	Mean	0.51750	0.01031	± 0.02866
	Stand. Dev.	0.02062		
	G1	-0.71333	1.01418	± 1.98820
	G2	1.78546	2.61861	± 5.13351
	K-S Dmax	0.20174		
IIIB	Mean	0.41786	0.00806	± 0.01726
	Stand. Dev.	0.03017		
	G1	-0.44682	0.59738	± 1.17110
	G2	1.19817	1.15405	± 2.26239
	K-S Dmax	0.18299		

Table 1 -- Continued

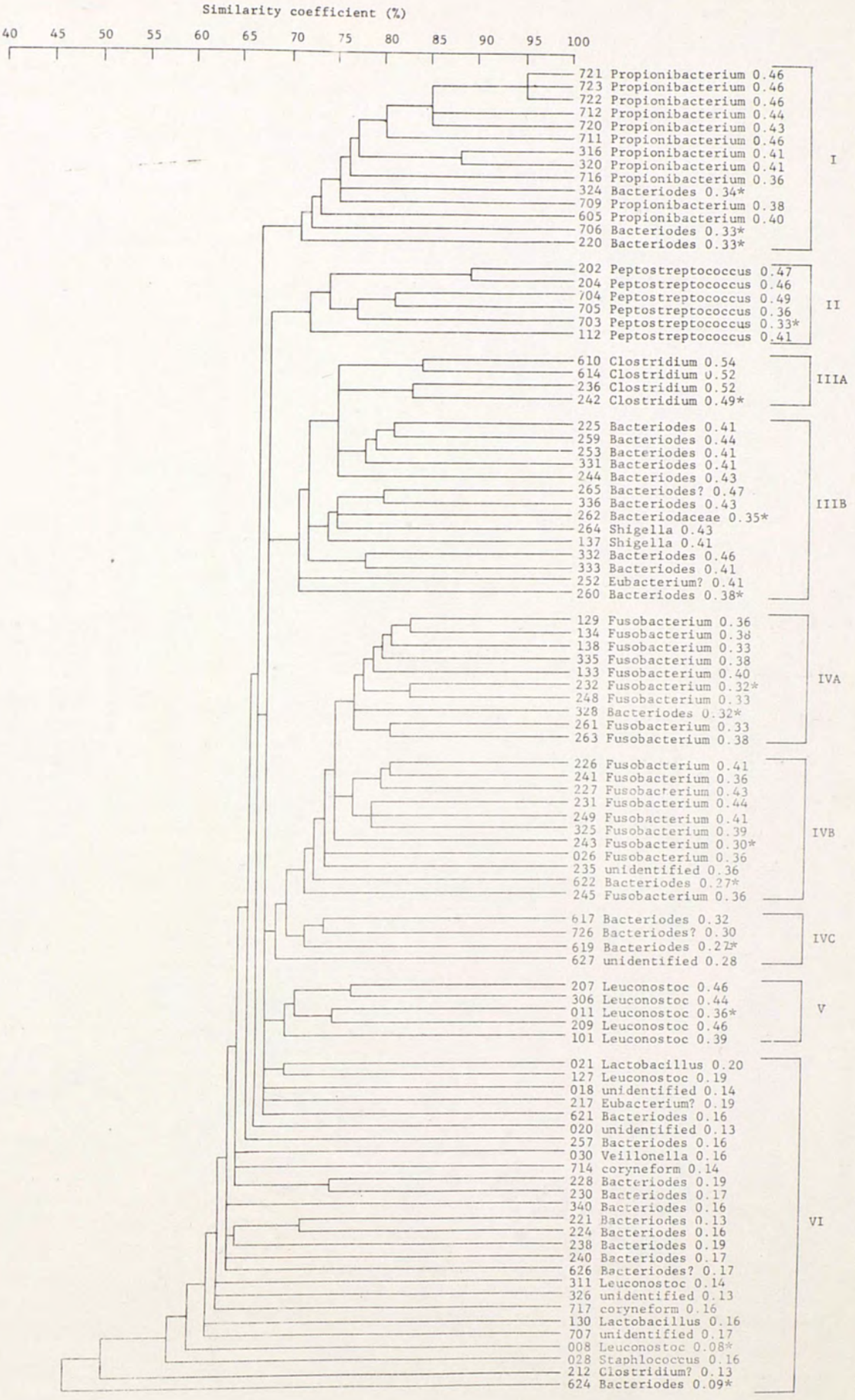
Phenon		Statistic	Stand. Error	95% Confid. Limits
IVA	Mean	0.35300	0.00955	± 0.02130
	Stand. Dev.	0.03020		
	G1	0.27645	0.68704	± 1.34688
	G2	-1.79840	1.33425	± 2.61565
	K-S Dmax	0.27682		
IVB	Mean	0.37182	0.01577	± 0.33470
	Stand. Dev.	0.05231		
	G1	-0.68422	0.66069	± 1.29521
	G2	0.03453	1.27942	± 2.50816
	K-S Dmax	0.13790		
IVC	Mean	0.29250	0.01109	± 0.03082
	Stand. Dev.	0.02217		
	G1	0.48157	1.01418	± 1.98820
	G2	-1.69950	2.61861	± 5.13352
	K-S Dmax	0.21353		
V	Mean	0.42200	0.02010	± 0.05166
	Stand. Dev.	0.04494		
	G1	-0.71375	0.91287	± 1.78959
	G2	-1.84247	2.00000	± 3.92078
	K-S Dmax	0.19892		
VI	Mean	0.15500	0.00569	± 0.01167
	Stand. Dev.	0.02902		
	G1	-0.87261	0.45556	± 0.89307
	G2	1.01717	0.88651	± 1.73791
	K-S Dmax	0.18379		

Note: Statistics have been reported without truncation as generated by the computer.

Table 2. Critical values one standard deviation from the mean of each phenon. Organisms in Fig. 1 with I_{RS} values less than these values for each respective phenon are considered to be poor members of that phenon.

Phenon	Critical values
I	0.355
II	0.356
IIIA	0.497
IIIB	0.388
IVA	0.323
IVB	0.320
IVC	0.270
V	0.377
VI	0.126

Fig. 1. Phenogram of isolates from a sanitary landfill (preceded by strain numbers) and their associated Index of Relevance and Similarity (I_{RS}) values. Organisms greater than one standard deviation from the mean of their respective phena are judged to be poor members of those phena and are designated (*).


Similarity coefficient (%)
40 45 50 55 60 65 70 75 80 85 90 95 100
721 Propionibacterium 0.46
723 Propionibacterium 0.46
722 Propionibacterium 0.46
712 Propionibacterium 0.44
720 Propionibacterium 0.43
711 Propionibacterium 0.46
316 Propionibacterium 0.41
320 Propionibacterium 0.41
716 Propionibacterium 0.36
324 Bacteriodes 0.34*
709 Propionibacterium 0.38
605 Propionibacterium 0.40
706 Bacteriodes 0.33*
220 Bacteriodes 0.33*
I
202 Peptostreptococcus 0.47
204 Peptostreptococcus 0.46
704 Peptostreptococcus 0.49
705 Peptostreptococcus 0.36
703 Peptostreptococcus 0.33*
112 Peptostreptococcus 0.41
II
610 Clostridium 0.54
614 Clostridium 0.52
236 Clostridium 0.52
242 Clostridium 0.49*
IIIA
225 Bacteriodes 0.41
259 Bacteriodes 0.44
253 Bacteriodes 0.41
331 Bacteriodes 0.41
244 Bacteriodes 0.43
265 Bacteriodes? 0.47
336 Bacteriodes 0.43
262 Bacteriodaceae 0.35*
264 Shigella 0.43
137 Shigella 0.41
332 Bacteriodes 0.46
333 Bacteriodes 0.41
252 Eubacterium? 0.41
260 Bacteriodes 0.38*
IIIB
129 Fusobacterium 0.36
134 Fusobacterium 0.38
138 Fusobacterium 0.33
335 Fusobacterium 0.38
133 Fusobacterium 0.40
232 Fusobacterium 0.32*
248 Fusobacterium 0.33
328 Bacteriodes 0.32*
261 Fusobacterium 0.33
263 Fusobacterium 0.38
IVA
226 Fusobacterium 0.41
241 Fusobacterium 0.36
227 Fusobacterium 0.43
231 Fusobacterium 0.44
249 Fusobacterium 0.41
325 Fusobacterium 0.39
243 Fusobacterium 0.30*
026 Fusobacterium 0.36
235 unidentified 0.36
622 Bacteriodes 0.27*
245 Fusobacterium 0.36
IVB
617 Bacteriodes 0.32
726 Bacteriodes? 0.30
619 Bacteriodes 0.22*
627 unidentified 0.28
IVC
207 Leuconostoc 0.46
306 Leuconostoc 0.44
011 Leuconostoc 0.36*
209 Leuconostoc 0.46
101 Leuconostoc 0.39
V
021 Lactobacillus 0.20
127 Leuconostoc 0.19
018 unidentified 0.14
217 Eubacterium? 0.19
621 Bacteriodes 0.16
020 unidentified 0.13
257 Bacteriodes 0.16
030 Veillonella 0.16
714 coryneform 0.14
228 Bacteriodes 0.19
230 Bacteriodes 0.17
340 Bacteriodes 0.16
221 Bacteriodes 0.13
224 Bacteriodes 0.16
238 Bacteriodes 0.19
240 Bacteriodes 0.17
626 Bacteriodes? 0.17
311 Leuconostoc 0.14
326 unidentified 0.13
717 coryneform 0.16
130 Lactobacillus 0.16
707 unidentified 0.17
008 Leuconostoc 0.08*
028 Staphlococcus 0.16
212 Clostridium? 0.13
624 Bacteriodes 0.09*
VI

Discussion

Similarity coefficients have been used to determine the reliability of junction points in phenograms (5). Relevance values measure the reliability of the similarity coefficients. I believe that the product of the two gives a better measure of an operational taxonomic unit's position on the phenogram than either value alone. For example, it is quite possible that a compoaite organism generated from a phenon (sometimes referred to as the hypothetical mean organism) will have many characteristics which are scored as "no comparison" but that those characteristics which are scored otherwise will be shared by almost every member of the phenon. The similarity coefficients between the members and the composite may be high but the relevance value will be low. The I_{RS} value will be a modification of these two extremes.

The I_{RS} values in all the phena presented (Fig. 1) were normally distributed so the decision for the arbitrary critical point to separate good from poor members of the phena could easily be applied with uniformity. This reduced the temptation to force the data to agree with classical taxonomy.

Disparities such as the grouping of Shigella and Bacteriodes in phenon IIIB may imply a genuine relationship between the genera, at least to the extent of the characteristics used to generate the phenogram.

The I_{RS} values actually form a "gradient of fit" in the phenon and the decision to apply critical values to the gradient is arbitrary. Situations may exist where such values would be inappropriate or where a multiplicity of such values would ease analysis of the data.

The relatedness of the members of phenon VI is intuitively lower than that of the members of the other phena. It is possible that I_{RS} values could be used to show this statistically. If the mean values of the I_{RS} values of each phenon are grouped in a frequency distribution phena which fall a certain distance from this mean may be considered to be phena of poor internal relatedness.

The true worth of the I_{RS} can only be shown by further application of it to a variety of numerical taxonomic data and by more rigorous statistical analysis than was possible in this paper.

Appendix

This program is written in Fortran IV. It computes similarity coefficients using Jaccard's coefficient and arranges the coefficients in descending order. Data is coded as follows: 0 = negative, 1 = positive, 3 = no comparison. The program will accept up to 100 organisms and up to 100 bits of coded data for characteristics.

There are two types of input cards required: one input parameter card giving the number of organisms in the first three spaces of the card followed by the number of encoded bits of data per card in the second three spaces; and a variable number of data cards depending on the number of organisms. The first three spaces of the data card contains the numerical designation for each organism, the rest of the card up to and including space 72 contains the encoded data without spacing. If a second card is required to accomodate all the data per organism the data is continued beginning in the first space.

Test Data

Input

```
008050

00831000331031000031010103100000101111100010000100000
01131000331031000031010103100000100011301011111111111
01831000310010000010010101000000101111111011111111111
02010000310010000010031103310000103333311011111111111
02133100100010000031010101000000101111111011011010111
02633310100010000010031101000000311111011001000010101
02831000331031000033110103310000100111010001000111101
03010000331031000010010103333331310100010001111111101
```

Output

```
 8 31000331031000031010103100000101111100010000100000
11 31000331031000031010103100000100011301011111111111
18 31000310010000010010101000000101111111011111111111
20 10000310010000010031103310000103333311011111111111
21 33100100010000031010101000000101111111011011010111
26 33310100010000010031101000000311111011001000010101
28 31000331031000033110103310000100111010001000111101
30 10000331031000010010103333331310100010001111111101
85      18   20
76      18   21
72      11   18
66      11   20
61      20   21
        20   30
60      11   21
        21   26
58      11   28
57      28   30
54      11   30
53      18   26
        18   28
        18   30
50       8   11
47       8   28
45      26   28
44      21   28
42       8   18
41       8   21
39      26   30
38      21   30
32      11   26
25       8   30
23       8   26
19       8   20
```

```
C       COMPUTES PERCENT SIMILARITY USING JACCARD COEF
C       FICIENT AND ARRANGES COEFFICIENTS IN DESCENDING
C       ORDER WITH ASSOCIATED PAIRS OF ORGANISMS
C       CODED: 0=NEGATIVE, 1=POSITIVE, 3=NO COMPARISON
        INTEGER*2 IDATA (100,100),ID(100),ISMCO(100,100),
       AISORT(4900),NPT(4900)
C       IDATA STORES TWO TEST RESULTS, ID STORES ORGANISM
C       DESIGNATIONS, ISMCO STORES SIMILARITY COEFFICIENTS
C       ISORT IS USED WITH SUBPROGRAM TO ARRANGE COEF
C       FICIENTS IN ASCENDING ORDER, NPT STORES COEF
C       FICIENTS IN DESCENDING ORDER
        DO 9 K=1,4900
        ISORT(K)=0
9       NPT(K)=0
        DO 101 JJ=1,100
        DO 101 II=1,100
101     IDATA(JJ,II)=0
        DO 100 I=1,100
        ID(I)=0
        DO 100 J=1,100
        ISMCO(I,J)=0
100     CONTINUE
C       NOSTR IS NUMBER OF STRAINS, NOATR IS NUMBER OF
C       ATTRIBUTES
1       READ (5,2) NOSTR, NOATR
2       FORMAT (2I3)
        IF(NOSTR+NOATR.EQ.0) GO TO 99
C       READ IN ORGANISM DESIGNATIONS AND ATTRIBUTES
        DO 50 K=1, NOSTR
        READ(5,3) ID(K), (IDATA(J,K),J=1,NOATR)
3       FORMAT(I3, 100I1)
        IF(ID(K).EQ.0) GO TO 98
        WRITE (6,51) ID(K), (IDATA(J,K)J=1,NOATR)
```

```
51      FORMAT (1X,I3,1X,100I1)
50      CONTINUE
C       COMPARE EACH ORGANISM WITH EVERY OTHER ORGANISM
        DO 44 J=1,NOSTR
        DO 44 I=J,NOSTR
        IF(I.EQ.J) GO TO 44
        SIML=0.0
        DIFF=0.0
        DO 55 M=1,NOATR
        ITOT=IDATA(M,I)+IDATA(M,J)
        IF(ITOT-3)6,55,55
6       IF(ITOT*1)55,7,8
7       DIFF=DIFF+1
8       SIML=SIML+1
55      CONTINUE
        IF(SIML+DIFF.LT.1.0)GO TO 97
C       COMPUTE SIMILARITY EXCLUDING NEGATIVE MATCHES
        ISMCO(J,I)=(SIML*100.0)/(SIML+DIFF)
        GO TO 44
97      WRITE(6,197)ID(J),ID(I)
197     FORMAT(' ','STRAINS',2I5,' HAVE NO SIM. OR DIFF')
44      CONTINUE
        NOTEMP=NOSTR-1
C       STORE SIMILARITIES IN ISORT TO PROCESS IN SUBROUTINE
        K=0
        NOPE=1
        DO 200 I=1,NOTEMP
        NOPE=NOPE+1
        DO 201 J=NOPE,NOSTR
        K=K+1
        ISORT(K)=ISMCO(I,J)
201     CONTINUE
200     CONTINUE
```

```
C       THIS IS A STANDARD SUBROUTINE
        CALL SORT(ISORT,1,K)
C       THIS REVERSES THE ORDER OF ISORT
        J=K+1
        DO 16 I=1,K
        J=J-1
        IF(J.EQ.0)GO TO 17
        NPT(I)=ISORT(J)
16      CONTINUE
17      CONTINUE
C       RE-ASSOCIATE ORGANISM DESIGNATIONS WITH SIMILARITY
C       COEFFICIENTS
        DO 10JJ=1,K
        NOPE=1
        DO 11 I=1,NOTEMP
        IDROW=ID(I)
        NOPE=NOPE+1
        DO 11 J=NOPE,NOSTR
        IDCOL=ID(J)
        IF(NPT(JJ).NE.ISMCO(I,J))GO TO 11
        IF(JJ.EQ.1)GO TO 15
        IF(NPT(JJ).NE.NPT(JJ-1))GO TO 15
        WRITE(6,13) IDROW, IDCOL
13      FORMAT(' ',5X,2I5)
        GO TO 14
15      WRITE(6,12) NPT(JJ),IDROW,IDCOL
12      FORMAT(' ',I3,2X,2I5)
14      ISMCO(I,J)=0
        GO TO 10
11      CONTINUE
10      CONTINUE
        GO TO 1
98      WRITE(6,198)
```

```
198     FORMAT(1X, 'BLANK STRAIN NUMBER FOUND')
99      STOP
        END
        SUBROUTINE SORT (A,II,JJ)
        DIMENSION A(4900), IU(20), IL(20)
        INTEGER*2 A,T,TT
C       THIS SORTS A FROM II TO JJ
C       IF IU, IL ARE OF DIMENSION K, SUBROUTINE WILL
C       SORT UP TO 2**(K+1)-U ELEMENTS
        M=1
        I=II
        J=JJ
5       IF(I.GE.J) GO TO 70
10      K=I
        IJ=(J+I)/2
        T=A(IJ)
        IF(A(I).LE.T) GO TO 20
        A(IJ)=A(I)
        A(I)=T
        T=A(IJ)
20      L=J
        IF(A(J).GE.T) GO TO 40
        A(IJ)=A(J)
        A(J)=T
        T=A(IJ)
        IF(A(I).LE.T) GO TO 40
        A(IJ)=A(I)
        A(I)=T
        T=A(IJ)
        GO TO 40
30      A(L)=A(K)
        A(K)=TT
40      L=L-1
```

```
      IF(A(L).GT.T) GO TO 40
      TT=A(L)
50    K=K+1
      IF(A(K).LT.T) GO TO 50
      IF(K.LE.L) GO TO 30
      IF(L-I.LE.J-K) GO TO 60
      IL(M)=I
      IU(M)=L
      I=K
      M=M+1
      GO TO 80
60    IL(M)=K
      IU(M)=J
      J=L
      M=M+1
      GO TO 80
      M=M-1
      IF(M.EQ.0) RETURN
      I=IL(M)
      J=IU(M)
80    IF(J-I.GE.11) GO TO 10
      IF(I.EQ.II) GO TO 5
      I=I-1
90    I=I+1
      IF(I.EQ.II) GO TO 70
      T=A(I+1)
      IF(A(I).LE.T) GO TO 90
      K=I
100   A(K+1)=A(K)
      K=K-1
      IF(T.LT.A(K)) GO TO 100
      A(K+1)=T
      GO TO 90
      END
```

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