To the Graduate Council:

I am submitting herewith a dissertation written by Lawrence Milton Mallory entitled "Numerical Taxonomic Analysis of Bacterial Guild Structure in Aquatic Environments." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology.

Gary S. Sayler, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

NUMERICAL TAXONOMIC ANALYSIS OF BACTERIAL GUILD STRUCTURE IN AQUATIC ENVIRONMENTS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Lawrence Milton Mallory

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ABSTRACT

Numerical taxonomic techniques and cellular fatty acid analysis were used to investigate the bacterial guild structure of aquatic environments. Physiological populations, as defined by isolation media, were examined. Classification techniques, both phenetic and chemotaxonomic, also were compared. A total of four isolation media were used: yeast extract, peptone, glucose and agar (YEPGA); lake water and agar (LWA); phenanthrene (a three ring polynuclear aromatic hydrocarbon), basal salts and agar (PHA); and polychlorinated biphenyls, basal salts and agar (PCBA). These media were chosen to isolate the following physiological populations respectively: high nutrient heterotrophs, low nutrient heterotrophs, heterotrophs resistant to phenanthrene and heterotrophs resistant to polychlorinated biphenyls.

A total of 210 bacterial strains was isolated using the four media from a single sample site located on an oligotrophic reservoir. The strains were examined for 158 morphological, physiological and biochemical characters. The results were coded and examined using numerical taxonomy. Including unclustered strains, a total of 100 taxa were characterized which indicated extremely high apparent diversity. When compared one to another, all four media were found to be somewhat selective in regards to taxa recovered. Taxa characterized from strains isolated from YEPGA and PHA were almost mutually exclusive. The difference could not be attributed solely to strain concentration in the water at the time of sampling.

Further studies using PHA as the isolation medium were carried out using sediment samples from a shallow freshwater stream. A total of 60 strains was isolated and examined using phenetic and chemotaxonomic methods. For the phenetic portion of the investigation the strains were characterized using 84 morphological, physiological and biochemical characters. The results were coded and examined using numerical taxonomy. Using methylated fatty acids and gas chromatography, cellular fatty acid profiles were obtained from the strains. These data also were coded and examined using numerical taxonomy.

In both the phenetic and the chemotaxonomic classifications over 80 percent of the bacterial strains were recovered in groups. Totals of 12 and 11 groups were defined in the phenetic and chemotaxonomic classifications respectively. Both classification systems were good in that they were reproducible on a strain by strain basis, discriminating and not based on negative matching. Group to group correspondence of strains from one system to the other was not observed. To some extent inter- and intra- group similarities in both classification systems could be used to predict strain recovery from one system to the other.

It was suggested that the lack of congruence between the phenetic and chemotaxonomic classification systems could be due to the nature of the aquatic environment and the characteristics of bacteria adapted to that environment. The pattern of variation observed between the two systems could be explained by the presence of bacterial taxa with wide and varied physiological and biochemical capabilities, and short term adaptation of these bacterial taxa to the changing potential

roles present in the aquatic community. The physiological and biochemical variance of taxa, and the occurence of relatively diverse taxa within a phenetic group may be significant contributing factors to identification problems often encountered in microbial ecology and environmental microbiology.

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PART I: OVERVIEW OF NICHE AND SPECIES CONCEPTS

I. OVERVIEW

Heterotrophic bacteria fulfill three principle roles in aquatic ecosystems. These are: (i) nutrient cycling; (ii) breakdown of recalcitrant molecules, such as cellulose, lignin, chitin and man made compounds; and (iii) the facilitation of energy flow through the oxidation of reduced carbon compounds. Taken as a group, bacteria are active over a wide range of temperature (16, 78), pH (37, 5), salt concentrations (67), Eh (5) and pressures (111). They also are capable of utilizing a wide range of carbon compounds. Individual species, however, have much narrower ranges of growth. A population consists of a group of individuals of the same species within an environment. A bacterial guild consists of all bacterial populations present in a community. The diversity and concentration of bacterial populations present in an aquatic community (the bacterial guild structure) determine the adaptability of the community to environmental change, in terms of heterotrophic potential and nutrient cycling. The measurement of bacterial guild structure is an active area in microbial ecology (10, 49, 65, 72). Three distinct facets relating to bacterial guild structure should be examined. These are: (i) the nature of bacterial taxonomy, (ii) niche theory application as it relates to bacterial populations, and (iii) methods used to estimate bacterial niches and bacterial taxa.

Bacterial taxonomy is unique in many ways. Due to the simple morphology of most bacterial cells and colonies, morphological characters are of limited taxonomic importance. The small size of

bacteria, often close to the limits of resolution for light microscopy, and their simple morphology account for their poor fossil record (100). Thus bacterial taxonomy could not be practically based on cladistic relationships or morphological features. Recently, through the use of 16S ribosomal RNA sequence characterization, cladistic relationships between diverse groups of bacteria have been estimated (35). While highly significant, this technique is, as yet, too involved for routine examination of bacterial isolates. Bacterial taxonomists normally use measures of physiological growth ranges, biochemical activities as well as limited morphological observations in order to classify bacterial strains. Most of the major taxonomic divisions described for bacteria are made on the basis of physiological and biochemical data (17). In order to gather such data, living material in the form of populations acquired through the culture of a single cell is normally used as the operational taxonomic unit (OTU). In addition to the above data, chemotaxonomic and genetic data have been used to help classify bacteria (17, 21, 40, 42). No single type of data is considered to be both necessary and sufficient to classify bacteria (17, 100). However, phenetic data (morphological, physiological and biochemical) and genetic data (percent guanine + cytosine, DNA-DNA hybidizaion and RNA-DNA hybridization) are considered to be of major importance (17, 21, 100). A well characterized bacterial taxon will consist of a group of OTUs which are phenetically, chemotaxonomically and genetically similar. Investigations leading to the newly described genus Rhodococcus are excellent examples of a well defined bacterial taxon (39, 40, 41, 77, 105).

When bacterial cultures are recovered from natural environments such as freshwater lakes and streams, estuaries, oceans, fresh and salt water sediments, soils, and plant surfaces, classification and identification are often difficult (3, 10, 62, 71, 95). Reasons for this difficulty may include insufficient study, inappropriate methods, or the nature of the populations present.

The scientific concept of niche, as it is currently used, is relatively new. Grinnell first used the term in reference to the behavior, morphological adaptation and feeding habits of the California Thrasher (45). He implied that a species' niche could be used to describe the species' relationship in the community (45). He also clearly stated that no two species "regularly established in a single fauna" could exist having the same niche relationships (45). Other early investigators shifted the emphasis of the niche concept to the community level and related it to what was to become known as the commpetitive exclusion principle (33, 36, 47). The modern concept of niche, including the definition of fundamental and realized niche, and the relationship between an organism's niche and habitat were presented by Hutchinson (52). He mathematically described a niche as a multi-dimensional hypervolume describing a species' role or potential role in a community (52).

Bacteria are considered highly responsive to their environment (104). Slight changes in temperature, pH, available nitrogen, and carbon source availability and concentration may have a profound effect on the potential roles (available niches) for bacteria. Such environmental fluctuations would have a direct effect upon the bacterial guild within a community.

Numerical taxonomic techniques allow for simultaneous comparisons between a large number of OTUs over a large number of characters. Characters examined within a study typically include morphological, physiological and biochemical data (22, 71, 85, 100). Characters examined can be two-state, such as the presence or absence of an enzyme; quantitative multistate, such as temperature range; and qualitative multistate, such as color (100). Although not commonly examined, chemotaxonomic data, particularly complex data such as fatty acid methyl ester patterns, are well suited to numerical taxonomic examination (14, 32, 100).

Many of the concepts central to numerical taxonomy can be attributed to an 18th century French botanist, Michel Adanson (1727-1806) (100). The practical application of numerical taxonomy, however, did not occur until electronic computers became available. Modern numerical taxonomic studies date only from the late 1950s (73, 96, 97, 101). Sneath and Sokal (100) identify three major attributes which differentiate these studies from previous work. These are: (i) the equal weighting of characters, (ii) the use of many characters within a study, and (iii) the use of clustering techniques to build taxonomic structure. The first attribute, the equal weighting of characters, deserves additional comment. By treating all characters equally one does not presuppose the existence of any particular taxon. If one were to weight certain characters, such as the Gram stain or Kovac's oxidase test, then overall similarity within a group might be masked if such tests are of little value. By testing for a large number of characters (the second attribute) the information content per OTU is high.

Natural taxa, as defined by Sneath and Sokal (100), are thought to contain a high degree of information. The weighting of only a few characters might serve to distort such natural groups.

Since the the early 1950s numerical taxonomic techniques have been applied to a wide variety of studies (100). Numerical taxonomy has become an active discipline with applications in ecology (4, 109), social sciences (74), and even the arts and humanities (110). Because numerical taxonomic techniques are flexible, estimations of multi-dimensional hyperspace can be made using both quantitative and binary data. Thus, an estimation of a population's fundamental niche may be achieved by using physiological and biochemical data, such as those acquired during a bacterial taxonomic study. Certain common measures of similarity used in bacterial taxonomic studies can be directly related to measures of distance. The simple matching coefficient (S_{sm}) of Sokal and Michener (101) is such a coefficient. The square root of the complementary form of S_{sm} is equal to Euclidean distance based on unstandardized character states. With binary data, all OTUs would be described in a unit hypercube. It may be suggested that bacterial taxonomic structure and fundamental niche estimations are closely related.

PART II: NUMERICAL TAXONOMY OF BACTERIA FROM CENTER HILL RESERVOIR

I. INTRODUCTION

An aquatic microbial community is composed of an assemblage of highly diverse populations of eucaryotic and procaryotic microorganisms. The elucidation and definition of the structure of the bacterial component of communities within estuarine and oceanic ecosystems has been the focus of numerous investigations (10, 49, 65, 72). Such studies have demonstrated a wide diversity of phototrophic, chemolithotrophic, and heterotrophic aerobic and facultatively anaerobic microbial populations in the water column of aquatic ecosystems. These bacterial populations are found to colonize three general habitats within the water column of an aquatic environment. The three general habitats are: (i) freeliving unattached bacterial populations (planktonic), (ii) bacteria attached or associated with detrital material (sestonic), and (iii) bacteria attached or associated with other living organisms (epiphytic). Although each habitat may support unique bacterial populations, the habitat itself is not sufficiently specific to define characteristic bacterial populations. Regardless of the habitat colonized, the fundamental role of these bacterial populations within the ecosystems is transformation and cycling of energy and matter. The efficiency of these transformations relates directly to niche specialization by the microorganisms and environmental perturbations.

In recent years considerable interest has arisen concerning the role of these microbial populations in the transformation (biodegradation) of environmental contaminants, the impact of environmental

contaminants on microbial processes and the utility of microbiological assessment in examining the occurrence and fate of environmental contaminants. As a result, techniques for the elucidation of microbial populations capable of transforming environmental contaminants are abundant. However, little attention has been given to the significance of the technique or the microbial populations enumerated with respect to their relationship to the microbial community and the niches those organisms occupy.

Numerical taxonomic techniques have been used to examine the relationships among specific groups of aquatic bacteria (1, 9, 21, 84) as well as highly diverse bacterial populations (3, 61, 62, 71, 80, 95). It was hypothesized that numerical taxonomy could aid in describing the niches of natural bacterial populations and microbial response to environmental contaminants. The principle goal of this investigation was to characterize the aerobic and facultative anaerobic portions of the heterotrophic aquatic bacterial guild structure in the photic zone of an oligotrophic freshwater environment. This community would be expected to be responsive to environmental contaminants as judged from previous studies reporting biodegradative and resistant bacteria isolated from aquatic environments. The specific objectives of this study were three-fold and included: (i) establishing the comparative relationship among biodegradative/resistant bacterial populations, recovered from selective media, to total culturable heterotrophs enumerated on low and high nutrient media; (ii) defining phenetic groups and estimating bacterial niche structure based on the physiological and biochemical diversity of taxonomic characteristics of the

bacterial guild within the aquatic community; and (iii) examining the limitations associated with both isolation and characterization of aquatic bacterial populations within the framework of numerical taxonomy.

II. MATERIALS AND METHODS

Recovery and Culture of Operational Taxonomic Units

Sample Collection

Reservoir samples were collected from site 4, Center Hill Reservoir in central Tennessee in January, 1977 (Fig. 1). This oligotrophic reservoir has been previously described (88) and studies have been conducted on the biodegradation of polychlorinated biphenyls (PCB) (92) and polyaromatic hydrocarbons (91) as well as the effects of these contaminants on heterotrophic microbial populations and processes (88). Physical and chemical sample characteristics at the time of sampling are given in Table 1. The reservoir was sampled using a Niskin Sterile Bag Sampler (General Oceanics, Miami, Fla.). A 3 liter pooled sample was maintained at ambient water temperature during transport and was processed at the field station facilities within 1 hour of initial sampling.

Operational Taxonomic Unit Isolation and Subculture

Microbial populations were enumerated and isolated using the spread plate technique following a ten-fold dilution series in quarter strength Ringers buffer. Four media were inoculated in triplicate over the dilution range using a 0.1 ml sample inoculum. The media employed include a moderately high nutrient yeast extract-peptone-glucose agar (YEPGA) (88); low nutrient, lake water agar (LWA) formulated with 1.8 percent (w/v) purified agar in aged Center Hill Reservoir water; phenanthrene agar (PHA) (88) consisting of basal salts, yeast extract,

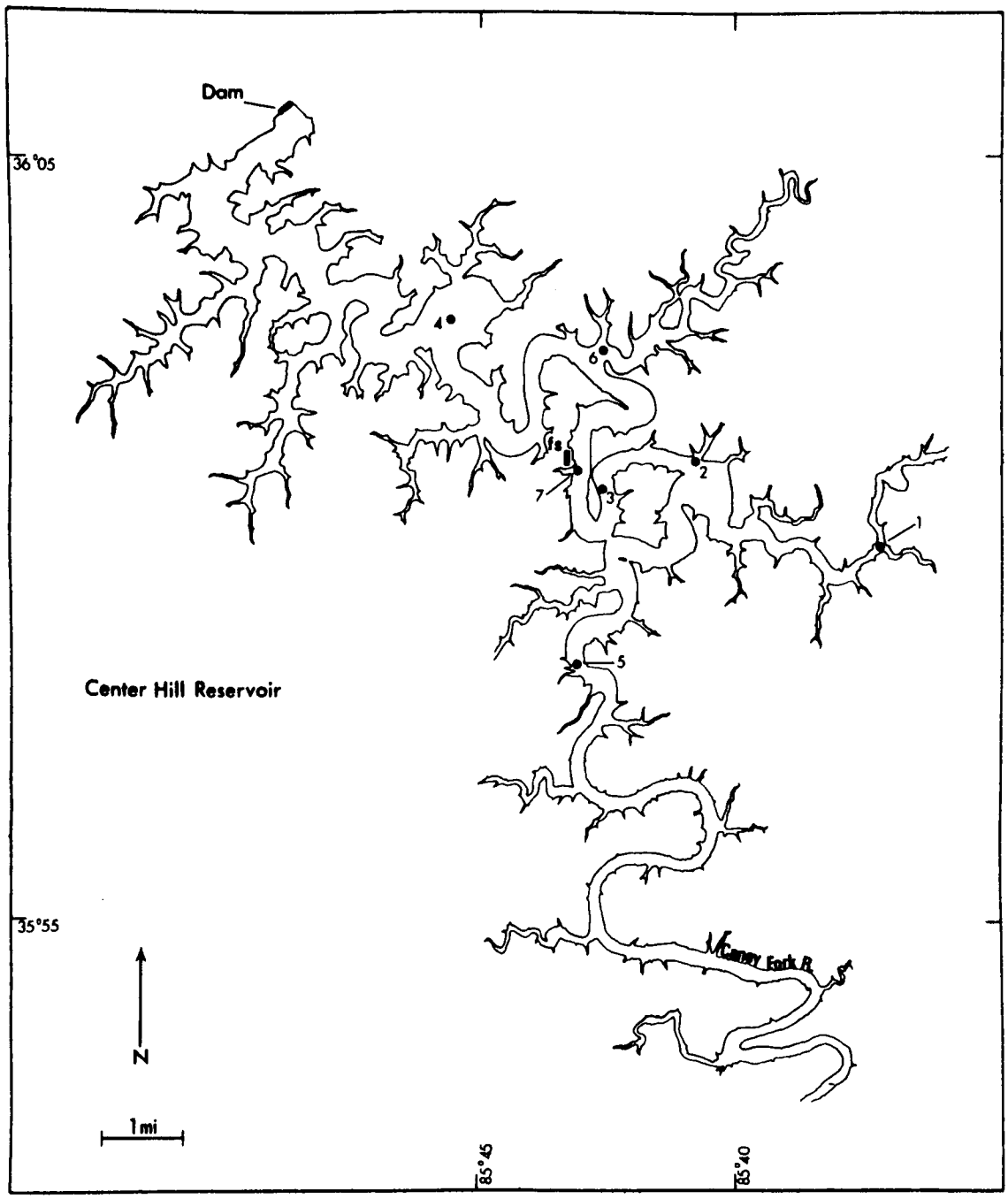


Figure 1. Sample site locations on Center Hill Reservoir, Tennessee. Field station (fs) facilities are located adjacent to site 7.

Table 1. Physical and chemical parameters of site 4 at the time of sampling

Variable	Measured Value
Water temperature	4.5°C
Transparency	4.0 m
Dissolved organic carbon	50 mg/1
Phosphate	18 ug/1
Nitrate	920 ug/1
Dissolved oxygen	12.5 mg/1
Conductivity	100 umohs
pH	7.75
Suspended sediments	1.3 mg/1

1.8 percent (w/v) purified agar, and 1.0 g l^{-1} certified phenanthrene (Eastman Organic Chemicals, Rochester, N.Y.) in distilled water; and polychlorinated biphenyl agar (PCBA) (88) identical to PHA except that phenanthrene was replaced with 1.0 g l^{-1} Aroclor 1254 (Monsanto Chemical Co., St. Louis, MO), a mixture of polychlorinated biphenyls, 54 percent chlorine by weight. Inoculated plates were incubated for 4 weeks at 25°C in the dark.

Following incubation, 240 well isolated colonies were randomly chosen for study. Sixty colonies were picked from each medium. Each isolated strain, the operational taxonomic unit (OTU), was subcultured. Three successive isolation streaks were performed for each OTU. Purity was determined from uniform colonial morphology and from microscopic examination. OTUs were maintained on YEPGA slants. Fresh slants were inoculated every 8 weeks. A total of 28 OTUs was lost upon initial subculturing or shortly thereafter.

Reference Cultures

In addition to the strains already described, 13 reference cultures also were examined. These were Alcaligenes dispar (American Type Culture Collection [ATCC] 3121), Bacillus cereus var. mycoides (ATCC 6462), Bacillus subtilis (ATCC 6051), Erwinia herbicola (ATCC 12287), Erwinia carnegieana (ATCC 13452), Erwinia carotovora (ATCC 8061), Escherichia coli (ATCC 11775), Klebsiella aerogenes (National Collection of Type Cultures 8172), Micrococcus luteus (ATCC 4698), Moraxella phenylpyruvica (ATCC 23333), Nocardia cariarum (ATCC 14629), Nocardia corallina (ATCC 4273) and Streptomyces griseus (ATCC 23345).

OTU Characterization

General Test Conditions and Controls

The OTUs were examined for 158 morphological, physiological and biochemical characters. Test media were inoculated and incubated at 25°C for 4 weeks unless otherwise noted. In order to assess test reproducibility 20 OTUs were examined in duplicate. Tests were carried out once and were repeated only when inconclusive results were obtained.

Morphology and Staining Reactions

Colonial morphology data were recorded following 7 days incubation on YEPGA. Selected characters from Colwell and Wiebe (22) were used. Fluorescent pigment production was determined using medium B of King et al. (64). Motility and cellular morphology were determined from wet mounts. The wet mounts were prepared from 2 to 3 day old broth cultures incubated at 25°C and examined by phase microcopy. Gram staining reactions of 2 to 3 day old broth cultures were determined using Hucker's modification of the Gram stain (50). Acid fastness was tested using 5 day old cultures by the method described by Cowen and Steel (26). A decolorizer of 5 percent (w/v) H_2SO_4 was used. Intracellular granulation was detected in 2 to 3 day old cultures using a methylene blue stain (6). Gliding motility was determined using the method by Colwell and Wiebe (22).

Physiological Characters

Growth in the presence of NaCl (2 percent, 5 percent 7.5 percent, 10 percent, 12.5 percent and 15 percent all w/v) was tested in NaCl amended medium YEPGA. Temperature growth range was

determined using YEPGA medium. Plates were lightly inoculated and incubated at 4°C, 15°C, 37°C and 42°C for 4 weeks, 2 weeks, 5 days and 3 days respectively. Visible growth was scored as a positive result. Resistance to the following dyes and other inhibitors were determined by using appropriately amended YEPGA: 0.00001 percent (w/v) each of brilliant green, crystal violet, malachite green, methylene blue, neutral red and pyronin B; 0.02 percent (w/v) of both bismuth citrate and potassium tellurite; and 0.04 percent (w/v) sodium azide. Any growth was scored as a positive test.

Biochemical Characters

The production of arginine dehydrolase and lysine and ornithine decarboxylase was determined using the method of Moeller (75). The arginine dehydrolase and lysine and ornithine decarboxylase tests were read at 2 and 4 days incubation and 2 and 4 weeks incubation. The methyl red test, indol production and acetoin production were determined following 5 days incubation at 25°C (6). The presence of oxidase (6) and catalase (6) was tested using fresh, newly visible growth from YEPGA. Nitrate and nitrite reduction were determined using the method described by Bailey and Scott (6). The production of H_2S was determined in SIM medium (Difco). Levan production was tested using YEPGA amended with 5 percent (w/v) sucrose. Copious slime production was scored as a positive test. The fermentation of glucose was tested using the method of Hugh and Liefson (51). Under aerobic conditions, acid production from the following carbohydrates was examined: L+ arabinose, cellobiose, dextrin, glucose, inositol, lactose, maltose, melezitose, melibiose, alpha methyl glucoside, raffinose, salicin,

sucrose and xylose. Where possible the carbohydrates were sterilized by filtration, otherwise sterilization was performed by successive heating to 100°C for 1 hour on 3 consecutive days. The sterilized basal medium of Hugh and Liefson (51) was mixed with a carbohydrate and aseptically dispensed into sterile multidishes. The media were inoculated with a multipoint inoculator (69), and were read at 1, 2, 4, 7 and 14 days. The degradation of hypoxanthine, starch and tyrosine was tested using YEPGA amended with 0.5 percent (w/v), 1.5 percent (w/v) and 0.5 percent (w/v) amounts respectively. Starch degradation was confirmed by flooding the plate with a dilute iodine solution. Clear zones around colonies were scored as positive. For the insoluble compounds, clearing of the medium around colonies was scored as a positive result. Agar degradation was determined using the method of Colwell and Wiebe (22). Casein degradation was tested by incorporating 5 percent (w/v) skim milk into YEPGA. Clearing of the medium was scored as a positve test. The degradation of DNA and urea was carried out using the methods of Bailey and Scott (6). Tween 20, Tween 40, Tween 60 and Tween 80 degradation was tested using the method of Sierra (94). The precipitation of calcium salts around areas of bacterial growth was taken to be a positive result. Egg yolk agar was prepared using 5 percent (v/v) egg yolk in YEPGA. Clearing of the medium around areas of growth was scored as positive.

Utilization of sole carbon sources was tested using the method of Stevenson (102). Carbon sources were formulated in 20 percent (w/v) solutions and sterilized as previously described. The solutions were added to the support medium to give a final concentration of 0.2 percent

(w/v). The complete media were aseptically dispensed into sterile replidishes and inoculated using multipoint inoculators (69). A total of 50 carbon sources was tested. These were sodium acetate, adonitol, L- alanine, L+ arabinose, L+ arginine, sodium aspartate, sodium benzoate, betaine, 1 - butanol, sodium butyrate, D+ cellobiose, sodium citrate, L- cystine, dulcitol, sodium formate, D- fructose, D- galactose, D- glucose, sodium glutamate, glycerol, glycogen, inositol, inulin, sodium lactate, lactose, L+ lysine, malonic acid, maltose, mannitol, D+ mannose, D+ melezitose, melibiose, sodium oxalate, L+ ornithine, proline, 1 - propanol, sodium pyruvate, alpha L rhamnose, D- ribose, salicin, salicylic acid, octanoic acid, DL- serine, sorbitol, sodium succinate, sucrose, alpha alpha- trehalose, DL- tryptophan, uracil and D- xylose. Both positive and negative controls were included. Results were recorded after 2 and 4 weeks incubation.

OTU Classification

Coding of the Data

The characters were coded into three states: "1" for a positive test, "0" for a negative test, and "9" for a noncomparable or not applicable test. Quantitative multistate characters, such as NaCl tolerance, were coded in a nonadditive fashion. The final n x t matrix contained 225 OTUs and 158 coded characters.

Computer Analysis

The data were examined using both the Jaccard coefficient (S_J) (92), which considers only positive matches, and the simple matching coefficient (S_{sm}) (101), which takes into account both positive and

negative matches. Clustering was achieved using single linkage, unweighted average linkage (UPGMA) and weighted average linkage (WPGMA) techniques (100). Significant chaining was observed when the single linkage clustering technique was used. The results from the UPGMA and WPGMA were virtually the same. Only those results acquired from the UPGMA analysis are presented.

An inverted matrix (t x n) was generated and examined by using the S_{sm} and S_J similarity coefficients. Clustering was achieved by using the UPGMA technique. Using this method, character correlation was assessed. From the inverted matrix a smaller matrix was generated for carbon compounds which were used in both the sole carbon source tests and the acid production tests. This smaller matrix was examined in an identical manner as the larger inverted matrix.

The programs used included the TAXAN 5 and IGPS 3 program packages obtained from Dr. R. R. Colwell at the University of Maryland. The programs were rewritten for the Dec System 10 computer.

III. RESULTS

Classification and Group Characteristics

Group Definition

A total of 153 OTUs and 1 reference strain comprising 72 percent of the total OTUs were clustered in 41 phenetic groups defined at the 80 to 85 percent similarity level using the S_{sm} coefficient (Fig. 2A-D). Examination of the OTUs using the S_J coefficient produced clusters essentially identical in membership to those obtained using the S_{sm} coefficient. Certain strains, e.g. B 58, B 7 and B 45, were shown to cluster above 80 percent similarity but were not defined in any groups. When examined using the S_J coefficient these strains were not recovered in clusters. The high level of similarity observed with the S_{sm} coefficient for these three OTUs was found to be attributable to a high degree of negative matching.

A simplified dendrogram (Fig. 3) showing defined clusters while omitting single strains exhibits each cluster's defined similarity level and its relative size. Three clusters (2, 5 and 40) containing 8 or more strains comprise 23 percent of the clustered OTUs. There were 27 small clusters with 2 to 3 members each. These OTUs accounted for 41 percent of grouped strains. The remaining 11 clusters with 4 to 7 members contained 36 percent of the clustered OTUs.

General Characteristics of the OTUs

The percent positive response to the coded characters used in this study are given in Table 2. The majority of positive responses

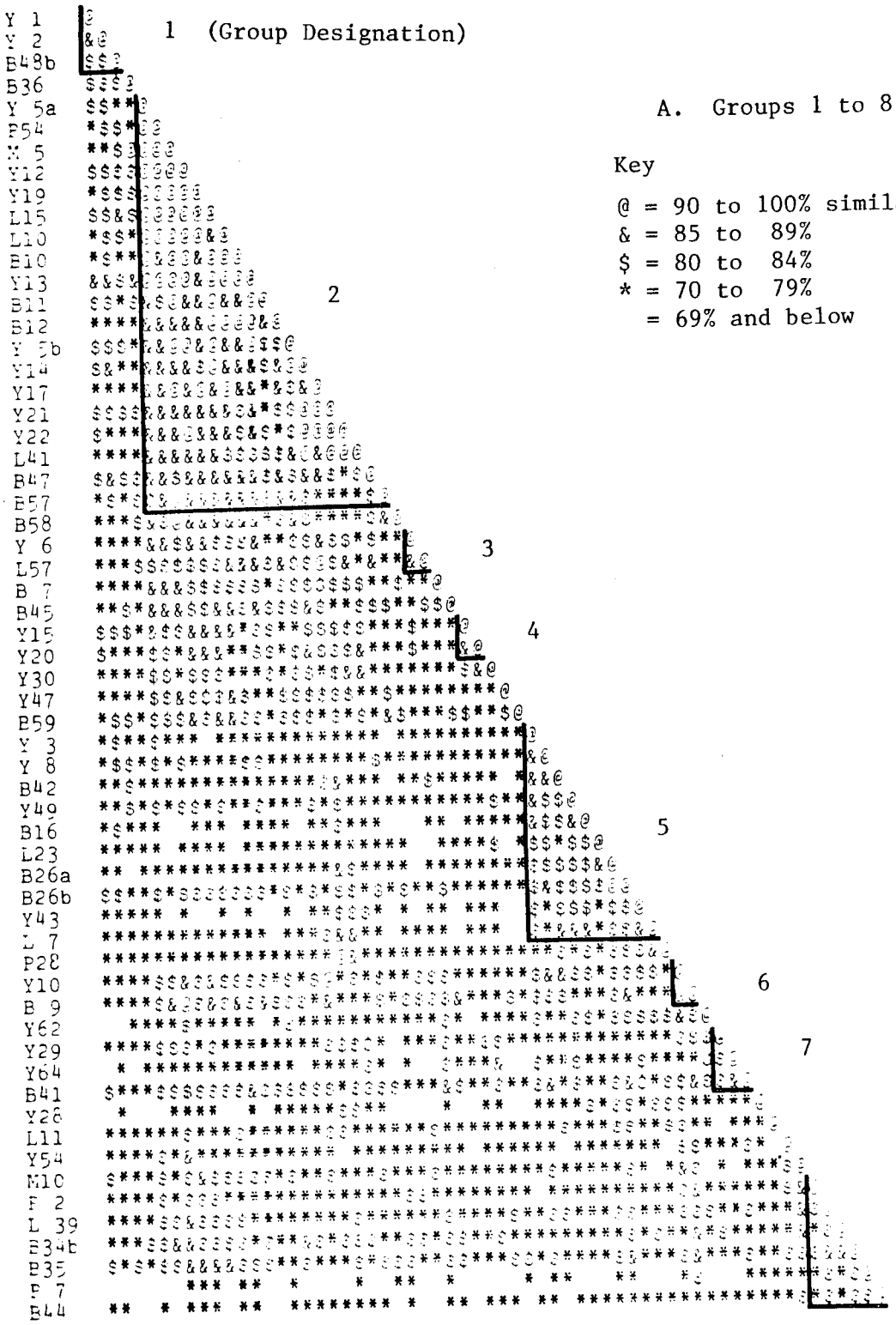


Figure 2. A sorted similarity matrix constructed using the S_{sm} similarity coefficient and the UPGMA clustering technique.

B. Groups 9 to 21

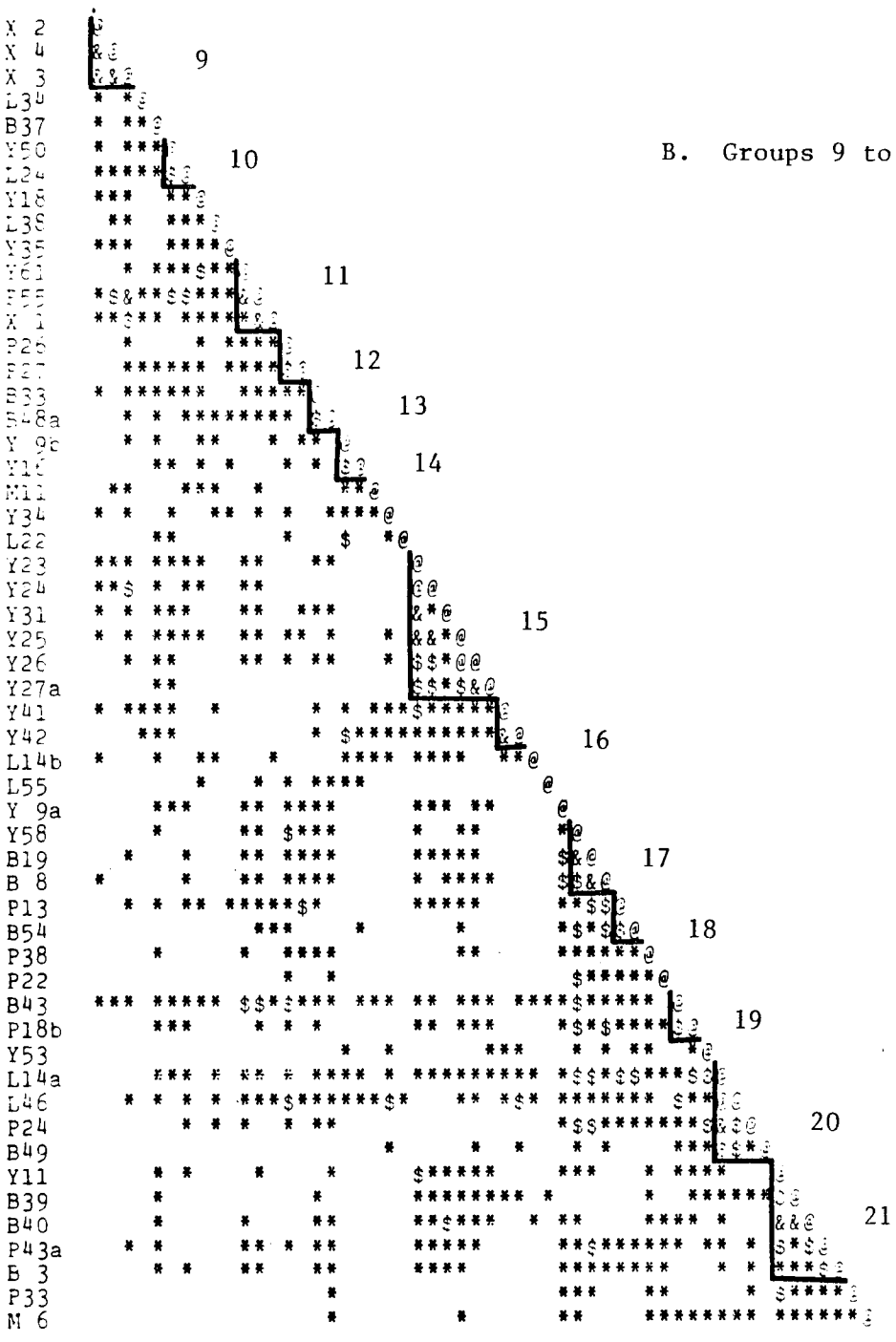


Figure 2. (Continued)

C. Groups 22 to 31

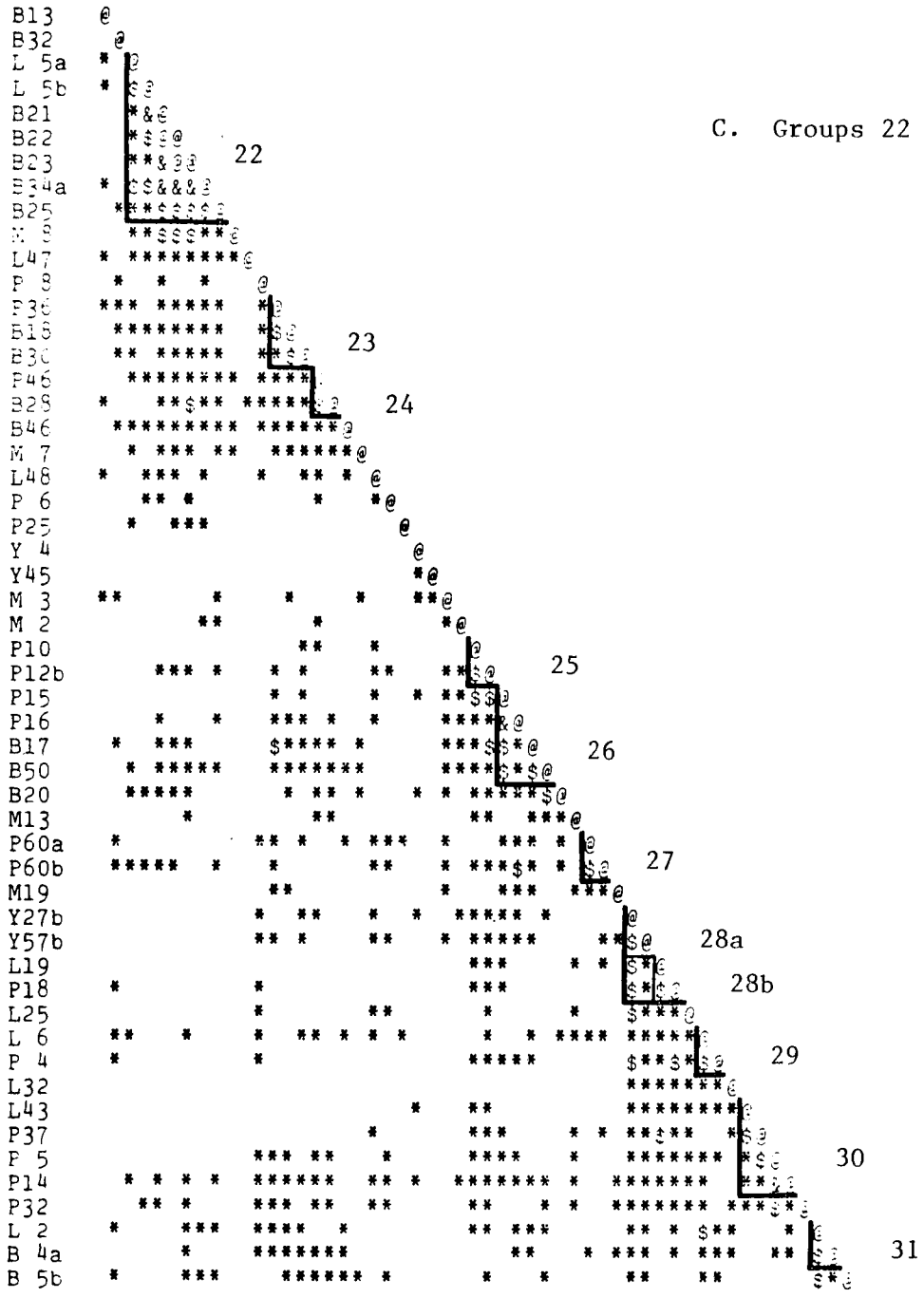


Figure 2. (Continued)

D. Groups 32 to 41

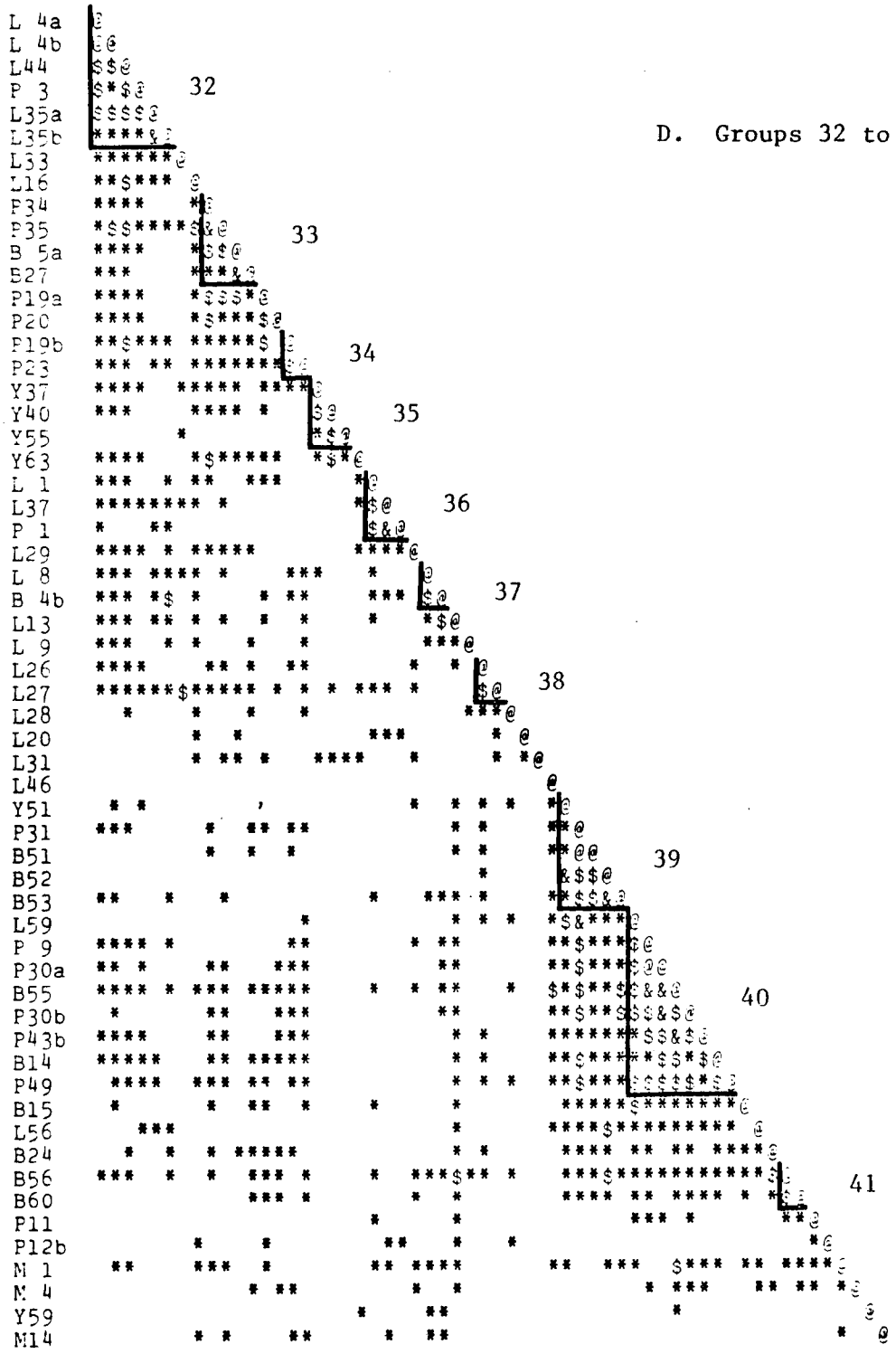


Figure 2. (Continued)

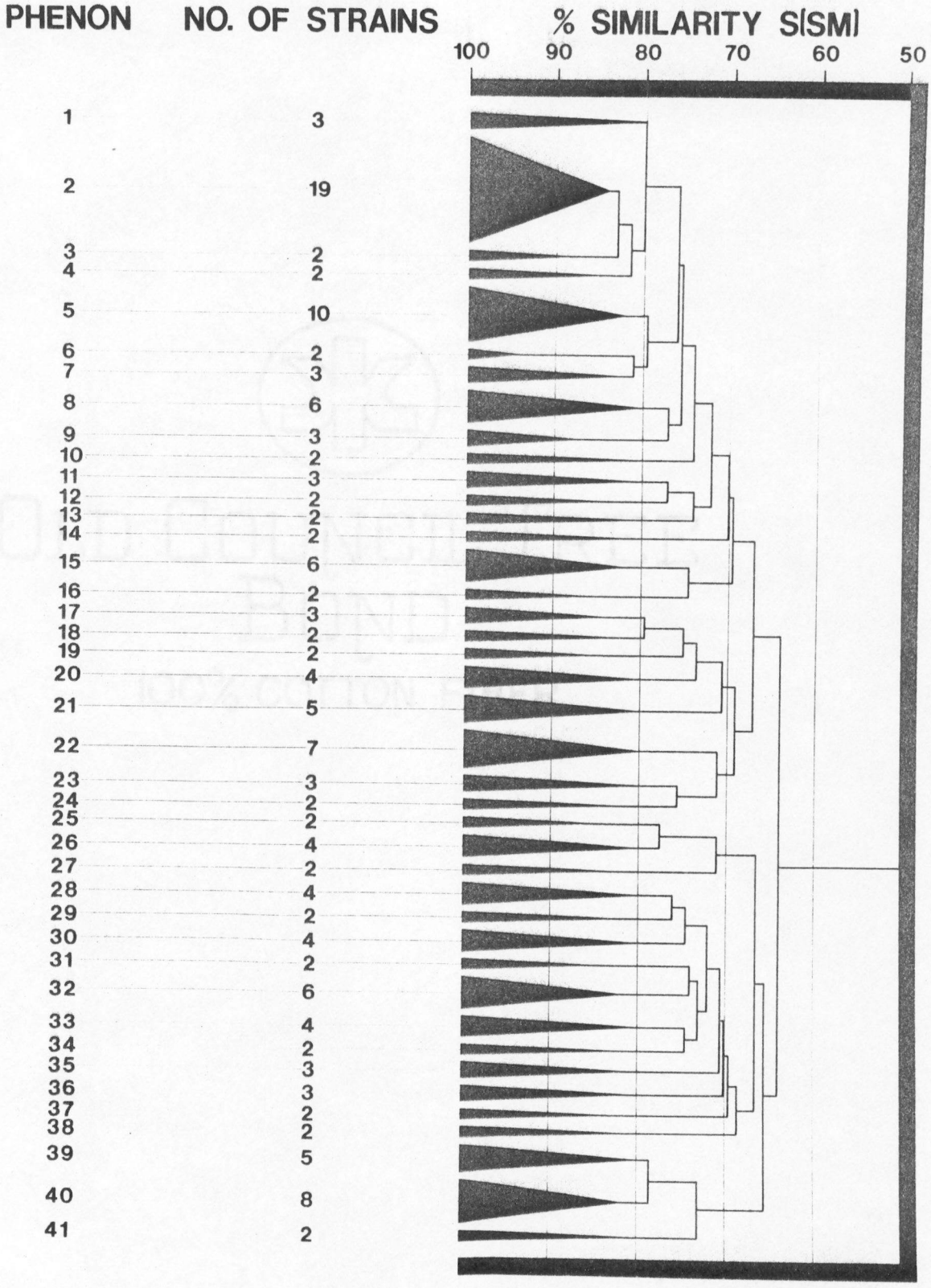


Figure 3. A simplified dendrogram of grouped Center Hill Reservoir OTUs constructed using the S_{sm} similarity coefficient.

Table 2. Percent positive frequency for characters used in the computer analysis

Character	Percent	Character	Percent
Colonial Morphology		chaining	8
elevation		rosette formation	3
convex	1	motility	
flat	3	gliding	3
lumpy	3	wet mount	33
peaked	10	Staining reactions	
raised	73	Gram positive	25
umbonate	10	Gram variable	15
edge		Gram negative	60
crenate	11	acid fast	1
irregular	13	granulation	10
smooth	76	Physiology	
surface		growth on NaCl containing media	
matt	1	2% (w/v) NaCl	50
shiny	69	5% " "	28
waxy	30	7.5% " "	8
transparency		10% " "	3
opaque	60	12.5% " "	1
translucent	32	initiation of growth at pH	
transparent	8	pH 5	63
color		pH 6	94
white	18	pH 9	95
off-white	29	growth at temperature	
light yellow	7	4°C	32
yellow	17	15°C	78
orange	18	37°C	40
pink	7	42°C	14
red	2	growth in the presence of	
purple	2	brilliant green	67
diffusible pigment		bismuth citrate	27
fluorescent pigment	4	crystal violet	68
Micromorphology		malachite green	63
length to width ratio		methylene blue	60
coccoid	4	potassium tellurite	11
1.5-3:1	38	pyronin B	64
3-6:1	72	sodium azide	1
7-15:1	29	Biochemistry	
15:1	18	acetoin produciton	5
cell characteristics		arginine dehydrolase	13
branching	4	catalase	60
round ends	99	indol production	2
tapered ends	5	levan production	7
stalked	3	lysine decarboxylase	9
curved	12	methyl red test	3
pleomorphic	8		
fragmenting	5		

Table 2. (Continued)

Character	Percent	Character	Percent
nitrate reduction	44	butanol	30
nitrite reduction	23	cellobiose	46
ornithine decarboxylase	3	cystine	12
oxidase	35	dulcitol	17
glucose metabolism		D fructose	63
alkaline reaction	15	D galactose	56
fermentative	35	D glucose	67
oxidative	35	glycerol	56
degradation of		glycogen	46
agar	3	inositol	32
casein	40	inulin	18
DNA	36	lactose	43
egg yolk	15	lysine	34
hypoxanthine	20	malonic acid	23
starch	24	maltose	58
Tween 20	43	mannitol	64
Tween 40	45	mannose	59
Tween 60	39	melezitose	29
Tween 80	34	melibiose	40
tyrosine	45	octanoic acid	18
urea	16	ornithine	42
acid production from		proline	52
adonitol	5	propanol	32
arabinose	28	rhamnose	37
cellobiose	29	ribose	46
dextrin	19	salicin	43
dulcitol	0	salicylic acid	1
glucose	44	sodium acetate	42
inositol	9	sodium aspartate	59
lactose	14	sodium benzoate	15
maltose	33	sodium butyrate	16
mannitol	25	sodium citrate	48
melezitose	9	sodium formate	18
melibiose	19	sodium glutamate	62
alpha methyl glucoside	6	sodium lactate	58
raffinose	9	sodium oxalate	13
salicin	8	sodium pyruvate	60
sucrose	26	sodium succinate	62
xylose	23	serine	23
utilization as sole		sorbitol	32
carbon source		sucrose	45
adonitol	24	trehalose	55
alanine	46	tryptophan	14
L+ arabinose	39	uracil	6
arginine	35	xylose	
betaine	34		

were associated with one character within each category of colonial morphology, with the exception of color. These characters were raised (73 percent), smooth edge (76 percent), shiny surface (69 percent) and opaque transparency (60 percent). Color tended to fall into two broad categories with approximately equal frequency. White and off-white accounted for 47 percent of the OTUs and light yellow, yellow and orange accounted for 42 percent.

The predominant micromorphological form observed was a rod with a length to with ratio of 3 to 1 to 6 to 1 (72 percent). Motility was observed in 36 percent of the OTUs. Only 3 percent exhibited gliding motility. Most OTUs, 60 percent, were Gram negative, 25 percent were Gram positive and 15 percent were Gram variable.

Tolerance of salt was low with 50 percent of the strains growing in the presence of 2 percent (w/v) NaCl. Only 28 percent grew in the presence of 5 percent NaCl. In marked contrast to this, the pH growth range was quite wide. When inoculated onto YEPGA pH 9, 95 percent of the OTUs initiated growth. At pH 6 and pH 5 the percent positive responses were 94 and 63 percent respectively. As one group the OTUs could be described as low temperature mesophiles with 78 percent of the strains growing at 15^{o}C. Nearly one third (32 percent) of the strains grew well at 4^{o}C. In the higher temperature range, 40 percent grew at 37^{o}C and only 14 percent grew at 42^{o}C. A surprising number of strains grew in the presence of dye. Growth in the presence of brilliant green, crystal violet, malachite green, methylene blue and pyronin B was 67 percent, 68 percent, 63 percent, 60 percent and 64 percent positive respectively.

Although most strains were capable of growth in the oxidation-fermentation test medium, only 35 percent gave a fermentative reaction while 65 percent gave either no reaction or an oxidative reaction. Many of the OTUs which were scored as oxidative exhibited a very weak reaction when compared to an uninoculated control. All other biochemical characters, with the exception of catalase and certain sole carbon source characters, had a positive response frequency of less than 50 percent.

The OTUs examined in this study were found to have a moderately diverse range of possible carbon sources. Of the 50 carbon compounds tested for utilization, 15 were used by 50 percent or more of the strains. The 15 carbon compounds included (i) nine sugars: D fructose (utilized by 63 percent of the strains), glucose (67 percent), glycerol (56 percent), D galactose (56 percent), maltose (58 percent), mannitol (64 percent), mannose (59 percent), trehalose (55 percent) and xylose (50 percent); (ii) three amino acids: proline (52 percent), sodium aspartate (59 percent) and sodium glutamate (62 percent); and (iii) three organic acids: sodium lactate (58 percent), sodium pyruvate (60 percent) and sodium succinate (62 percent). A total of 35 sole carbon sources was used by 30 percent or more of the OTUs examined.

Presumptive Identifications

Presumptive identifications were made for 24 groups. No identification could be made for 17 groups. Although group 2 contained the marker strain *Erwinia carnegieana*, no identification was made due to the highly atypical results obtained from that strain. The presumptive identifications are given in Table 3. Group characteristics are given in Tables 4, 5, 6, 7, and 8.

Table 3. Presumptive identifications

Group	Identification
1.	*Acinetobacter* sp.
2.	unidentified
3.	unidentified
4.	unidentified
5.	*Alcaligenes* sp.
6.	unidentified
7.	*Caulobacter* sp.
8.	*Streptothrix* sp.
9.	*Janthinobacterium* sp.
10.	unidentified
11.	*Pseudomonas* sp.
12.	unidentified
13.	unidentified
14.	unidentified
15.	*Flexibacter* sp.
16.	*Flexibacter* sp.
17.	*Corynebacterium* sp.
18.	coryneform
19.	*Aeromonas hydrophila*
20.	unidentified
21.	unidentified
22.	*Pseudomonas* sp.
23.	*Aeromonas hydrophila*
24.	*Pseudomonas* sp.
25.	*Kurthia* sp
26.	coryneform
27.	coryneform
28.	unidentified
29.	unidentified
30.	coryneform
31.	unidentified
32.	*Pseudomonas* sp.
33.	*Pseudomonas* sp.
34.	coryneform
35.	*Nocardia* sp.
36.	unidentified
37.	unidentified
38.	unidentified
39.	*Pseudomonas fluorescens*
40.	*Pseudomonas fluorescens*
41.	unidentified

Table 4. Characteristics of groups 1 to 10 from the Center Hill Reservoir OTUs

Character	Group Designation									
	1	2	3	4	5	6	7	8	9	10
Colonial Morphology										
elevation										
peaked	–[a]	–	–	–	–	–	–	–	–	+[b]
flat	–	d[c]	d	–	–	–	–	–	–	–
lumpy	–	–	–	–	–	–	–	–	–	–
raised	–	–	d	+	+	+	+	+	d	–
umbonate	+	d	–	–	–	–	–	–	d	–
edge										
crenate	–	–	–	–	–	–	–	–	–	–
irregular	–	–	–	–	–	–	–	–	+	–
smooth	+	+	+	+	+	+	+	+	–	+
surface										
matt	–	–	–	–	–	–	–	–	–	–
shiny	+	d	+	+	+	+	+	d	+	d
waxy	–	d	–	–	–	–	–	d	–	d
transparency										
opaque	d	d	–	d	d	–	–	+	+	+
translucent	–	d	+	–	d	+	+	–	–	–
transparent	d	d	–	d	–	–	–	–	–	–
color										
white	–	–	–	–	d	–	–	d	–	–
off-white	d	d	–	–	d	–	d	–	–	–
light yellow	d	d	d	–	–	–	–	–	–	–
yellow	–	d	d	+	–	–	–	–	–	d
orange	–	–	–	–	–	d	–	d	–	d
pink	–	–	–	–	–	d	d	d	–	–
red	–	–	–	–	–	–	–	–	–	–
purple	–	–	–	–	–	–	–	–	+	–
diffusible pigment										
fluorescent pigment	–	–	–	–	–	–	–	–	–	–
Micromorphology										
length to width ratio										
coccoid	–	–		–	–	d	–	–	–	–
1.5-3:1	d	–		–	+	d	d	d	d	+
3-6:1	+	d		+	+	+	+	d	+	d
7-15:1	d	d		–	–	d	d	d	–	–
15:1	–	–		–	–	–	–	+	–	–
cell characteristics										
branching	–	–		–	–	–	–	–	–	–
round ends	+	+		+	+	+	+	+	+	+
tapered ends	–	–		–	–	–	–	d	–	–
stalked	–	–		–	–	–	d	–	–	–
curved	d	–		–	d	–	–	d	d	–
pleomorphic	–	–		+	–	+	–	–	–	–
fragmenting	–	–		–	–	–	–	d	–	–

Table 4. (Continued)

Character	Group Designation									
	1	2	3	4	5	6	7	8	9	10
chaining	–	d		–	d	–	–	–	–	d
rosette formation	–	–		–	–	–	d	–	–	–
motility										
gliding	–	–	–	–	–	–	–	–	–	–
wet mount	d	d		–	+	–	d	–	+	–
Staining Reactions										
Gram stain[d]	–	–		–	–	var	–	–	–	–
acid fast	–	–	–	–	–	–	–	–	–	–
granulation	–	–		+	–	–	–	d	d	–
Physiology										
salt tolerance										
2% NaCl	d	–	+	–	d	d	–	d	–	–
5% NaCl	–	–	–	–	d	–	–	d	–	–
7.5% NaCl	–	–	–	–	–	–	–	d	–	–
10% NaCl	–	–	–	–	–	–	–	d	–	–
12.5% NaCl	–	–	–	–	–	–	–	–	–	–
initiation of growth at pH										
pH 5	–	–	–	–	–	–	–	+	–	–
pH 6	–	+	–	–	+	+	–	+	–	–
pH 9	+	d	–	–	+	+	d	+	+	+
growth at temperature										
4°C	d	–	–	d	–	–	–	–	d	–
15°C	d	d	d	d	d	+	d	d	d	d
37°C	–	–	–	–	d	–	–	–	d	d
42°C	–	–	–	–	–	–	–	–	d	–
resistance to dyes and other inhibitors										
brilliant green	d	–	–	–	+	+	+	+	+	+
bismuth citrate	+	d	–	–	–	–	–	d	–	–
crystal violet	+	–	–	–	+	+	+	+	+	+
malachite green	d	–	–	–	+	+	d	+	+	+
methylene blue	–	–	–	–	+	+	+	+	–	+
potassium tellurite	–	–	–	–	d	–	–	–	d	–
pyronin B	–	–	–	–	+	+	+	+	+	d
sodium azide	–	–	–	–	–	–	–	–	–	–
Biochemistry										
acetoin production	–	–	–	–	–	–	–	–	–	–
arginine dehydrolase	–	–	–	–	–	–	–	–	–	–
catalase	d	d	d	–	d	d	d	+	+	d
indol production	–	–	d	–	–	–	–	d	–	–
levan production	–	d	–	–	–	–	–	–	–	–
lysine decarboxylase	–	–	–	–	–	–	–	d	–	–
methyl red reaction	–	–	–	–	–	–	–	–	–	–
nitrate reduction	–	d	+	–	d	d	d	d	–	–
nitrite reduction	–	–	+	–	d	d	–	d	–	–

Table 4. (Continued)

Character	Group Designation 1	2	3	4	5	6	7	8	9	10
ornithine decarboxylase	-	-	-	-	-	-	-	-	-	-
oxidase	-	d		-	d	+	-	d	+	-
glucose metabolism										
alkaline reaction	-	-	-	-	d	-	d	-	d	-
fermentative	-	d	+	-	d	-	-	d	-	-
oxidative	d	d	-	-	d	-	d	d	d	-
degradation of										
agar	-	-	-	-	-	-	-	-	-	-
casein	+	-	-	d	-	-	-	d	d	-
DNA	d	-	-	-	d	-	d	d	-	-
egg yolk	+	-	-	-	-	-	-	-	-	-
hypoxanthine	-	-	-	-	-	-	-	-	+	-
starch	-	-	-	d	-	-	-	d	d	-
Tween 20	+	-	-	-	d	-	d	+	+	-
Tween 40	+	-	+	-	d	d	d	d	+	+
Tweem 60	+	-	-	-	-	+	d	d	+	d
Tween 80	+	-	-	-	-	-	d	d	+	-
tyrosine	+	d	-	-	d	-	-	d	+	-
urea	-	-	-	-	-	-	d	-	-	-
acid production from										
adonitol	-	-	-	-	-	-	-	-	-	-
arabinose	-	-	-	-	-	-	-	-	-	-
cellobiose	-	-	-	-	-	-	-	-	-	-
dextrin	-	-	-	-	-	-	-	d	-	-
dulcitol	-	-	-	-	-	-	-	-	-	-
glucose	-	d	-	-	-	-	-	d	-	-
inositol	-	-	-	-	-	-	-	-	-	-
lactose	-	-	-	-	-	-	-	-	-	-
maltose	d	-	-	-	-	-	-	-	-	-
mannitol	-	-	-	-	-	-	-	d	-	-
melezitose	-	-	-	-	-	-	-	-	-	-
melibiose	-	-	-	-	-	-	-	-	-	-
alpha methyl glucoside	-	-	-	-	-	-	-	-	-	-
raffinose	-	-	-	-	-	-	-	-	-	-
salicin	-	-	-	-	-	-	-	-	-	-
sucrose	-	-	-	-	-	-	-	d	-	-
xylose	-	-	-	-	-	-	-	-	-	-
use as sole carbon source										
adonitol	d	-	-	-	-	-	-	d	-	-
alanine	d	-	-	-	d	-	-	d	-	-
L+ arabinose	-	-	-	-	-	-	-	d	-	-
arginine	-	d	-	-	-	-	-	d	-	d
betaine	-	d	-	d	-	-	-	d	-	-
butanol	-	d	-	-	-	-	-	-	-	-
cellobiose	d	d	+	+	+	-	d	-	-	-
cystine	-	-	d	-	-	-	-	d	-	d

Table 4. (Continued)

Character	Group Designation									
	1	2	3	4	5	6	7	8	9	10
dulcitol	-	-	+	-	-	-	-	d	-	-
D fructose	+	d	+	d	d	+	-	-	-	-
D galactose	d	-	+	-	d	d	-	-	+	d
D glucose	+	-	+	+	+	+	-	-	d	d
glycerol	-	d	+	-	d	-	-	d	d	-
glycogen	-	d	d	-	d	-	-	-	-	-
inositol	-	-	d	-	-	-	+	d	-	-
inulin	-	d	-	-	d	-	-	d	-	d
lactose	d	d	-	-	d	-	d	d	-	+
lysine	-	-	-	d	d	-	d	-	-	+
malonic acid	d	-	d	-	d	-	-	d	-	-
maltose	d	d	d	-	d	-	d	d	d	-
mannitol	-	d	-	-	d	-	d	d	d	d
mannose	-	d	-	-	d	+	-	d	d	+
melezitose	-	-	-	-	-	-	-	-	d	-
melibiose	-	-	d	-	-	-	d	d	-	d
octanoic acid	-	-	-	-	-	-	-	-	-	-
ornithine	-	-	-	+	d	-	+	d	d	+
proline	d	d	-	-	d	-	+	d	-	d
propanol	-	-	-	-	d	-	+	d	-	+
rhamnose	-	d	-	+	d	-	-	-	-	d
ribose	-	-	+	-	+	-	-	d	-	+
salicin	d	d	-	+	d	-	d	d	-	-
salicylic acid	-	-	-	-	-	-	-	-	-	-
sodium acetate	d	d	-	-	d	-	-	d	-	-
sodium aspartate	d	d	-	d	d	-	-	d	-	-
sodium benzoate	-	-	-	-	-	-	-	-	-	-
sodium butyrate	-	-	-	-	-	-	-	-	-	-
sodium citrate	d	-	-	-	d	-	d	d	-	-
sodium formate	-	-	-	+	-	-	-	-	-	-
sodium glutamate	d	d	-	-	d	-	-	-	d	-
sodium lactate	-	d	-	d	d	-	d	d	d	+
sodium oxalate	-	-	-	-	-	-	-	-	d	-
sodium pyruvate	d	d	-	+	d	-	+	d	-	d
sodium succinate	+	d	-	d	d	-	+	d	+	+
serine	-	-	-	d	-	-	d	-	-	d
sorbitol	d	-	+	-	-	-	-	d	d	-
sucrose	d	-	-	+	d	-	d	-	-	+
trehalose	d	d	-	+	d	-	d	d	d	d
tryptophan	-	-	-	-	-	-	-	-	-	-
uracil	-	-	-	-	-	-	-	-	-	-
xylose	-	-	-	-	d	-	d	-	-	d

[a]- = less than 10 percent positive.

[b]+ = 90 to 100 percent positive.

[c]d = 10 to 89 percent positive.

[d]For the Gram stain only, - = Gram negative and var = Gram variable.

Table 5. Characteristics of groups 11 to 20 from the Center Hill Reservoir OTUs

Character	Group Designation									
	11	12	13	14	15	16	17	18	19	20
Colonial Morphology										
elevation										
peaked	–[a]	–	–	–	–	–	–	–	–	–
flat	–	–	–	–	–	–	–	–	–	–
lumpy	–	–	–	–	–	d[b]	–	–	–	–
raised	+[c]	+	+	+	+	d	+	+	+	d
umbonate	–	–	–	–	–	–	–	–	–	d
edge										
crenate	–	–	–	–	d	–	–	–	–	–
irregular	–	–	d	–	d	d	–	–	–	–
smooth	+	+	d	+	d	d	+	+	+	+
surface										
matt	–	–	–	–	–	–	–	–	–	–
shiny	d	+	+	+	d	+	d	+	–	d
waxy	d	–	–	–	d	–	d	–	+	d
transparency										
opaque	+	+	+	–	d	d	+	–	+	d
translucent	–	–	–	+	d	d	–	+	–	d
transparent	–	–	–	–	–	–	–	–	–	–
color										
white	–	–	–	–	–	–	–	–	–	–
off-white	–	d	–	d	–	d	–	–	–	–
light yellow	–	–	–	–	–	–	+	–	–	d
yellow	–	d	–	–	d	–	–	+	+	d
orange	d	–	+	d	d	d	–	–	–	–
pink	–	–	–	–	–	–	–	–	–	–
red	–	–	–	–	–	–	–	–	–	–
purple	d	–	–	–	–	–	–	–	–	–
diffusible pigment										
fluorescent pigment	–	–	–	–	–	–	–	–	–	–
Micromorphology										
length to width ratio										
coccoid	–	–	–	+	–	–	–	–	–	–
1.5-3:1	–	+	+	+	–	–	d	–	+	d
3-6:1	+	+	d	–	d	+	+	+	d	+
7-15:1	d	–	d	–	+	+	–	–	–	–
15:1	d	–	–	–	+	+	–	–	–	–
cell characteristics										
branching	–	–	–	–	–	–	–	–	–	–
round ends	+	+	+	+	+	+	+	+	+	+
tapered ends	–	–	–	–	–	–	–	–	–	–
stalked	–	–	–	–	–	–	–	–	–	–
curved	–	–	–	–	–	–	–	–	+	–
pleomorphic	–	–	–	–	–	–	–	–	–	–
fragmenting	–	–	–	–	–	–	–	–	–	–

Table 5. (Continued)

Character	Group Designation 11	12	13	14	15	16	17	18	19	20
chaining	-	-	-	-	d	-	-	-	-	-
rosette formation	-	-	-	-	-	-	-	-	-	-
motility										
gliding	-	-	-	-	d	+	-	-	-	-
wet mount	d	+	+	-	-	-	+	+	d	+
Staining Reactions										
Gram stain[d]	-	var	-	-	-	-	+	+	-	var
acid fast	-	-	-	-	-	-	-	-	-	-
granulation	-	-	-	-	-	-	d	-	-	-
Physiology										
salt tolerance										
2% NaCl	d	+	d	-	d	d	+	+	-	-
5% NaCl	d	d	-	-	-	d	d	+	-	-
7.5% NaCl	-	d	-	-	-	d	-	-	-	-
10% NaCl	-	-	-	-	-	d	-	-	-	-
12.5% NaCl	-	-	-	-	-	d	-	-	-	-
initiation of growth at pH										
pH 5	-	-	-	-	+	-	-	-	-	-
pH 6	+	+	-	-	+	-	+	+	-	-
pH 9	+	+	+	+	+	d	+	+	+	d
growth at temperature										
4°C	-	-	-	-	d	d	-	-	+	-
15°C	+	d	+	-	+	+	+	+	+	+
37°C	+	d	d	-	d	-	+	d	+	d
42°C	-	-	-	-	-	-	d	-	-	-
resistance to dyes and other inhibitors										
brilliant green	+	+	+	d	+	-	+	+	+	-
bismuth citrate	+	+	+	-	-	-	+	+	-	-
crystal violet	+	+	+	d	+	-	+	+	+	-
malachite green	+	+	+	-	+	-	+	+	+	-
methylene blue	+	+	+	d	+	-	+	+	+	-
potassium tellurite	-	-	-	-	d	-	-	-	-	-
pyronin B	+	+	+	-	+	-	+	+	+	-
sodium azide	-	-	-	-	-	-	-	d	-	-
Biochemistry										
acetoin production	-	-	-	-	-	-	-	-	-	-
arginine dehydrolase	-	-	-	-	-	-	-	-	-	-
catalase	+	+	+	-	d	d	+	+	-	-
indol production	-	-	-	-	-	-	-	-	-	-
levan production	d	-	+	-	-	-	-	-	-	-
lysine decarboxylase	-	-	-	-	-	-	-	-	-	-
methyl red reaction	-	-	-	-	-	-	-	-	-	-
nitrate reduction	-	-	-	-	d	+	-	+	-	-
nitrite reduction	-	-	-	-	d	+	-	d	-	-

Table 5. (Continued)

Character	Group Designation									
	11	12	13	14	15	16	17	18	19	20
ornithine decarboxylase	-	-	-	-	-	-	-	-	-	-
oxidase	d	+	-	-	d	-	d	d	-	-
glucose metabolism										
alkaline reaction	+	-	-	-	-	-	-	-	-	-
fermentative	-	d	-	-	d	+	+	+	+	+
oxidative	+	-	+	d	d	-	-	-	-	-
degradation of										
agar	d	-	-	-	-	-	-	-	-	-
casein	d	d	-	-	d	d	d	d	+	+
DNA	-	+	d	-	d	-	d	d	-	-
egg yolk	-	-	-	-	d	-	-	-	-	-
hypoxanthine	d	-	d	-	-	-	-	-	-	-
starch	-	-	-	-	+	+	-	-	d	-
Tween 20	+	-	+	d	d	-	d	d	d	-
Tween 40	+	d	d	-	+	d	d	d	d	-
Tween 60	+	-	-	-	d	d	d	d	d	-
Tween 80	d	-	-	-	d	+	-	d	d	-
tyrosine	+	+	d	-	+	-	+	+	d	-
urea	-	-	-	-	-		d	-	-	-
acid production from										
adonitol	-	-	-	-	-		-	-	-	-
arabinose	-	-	+	-	-		-	-	-	d
cellobiose	-	-	-	-	+		+	+	d	d
dextrin	-	-	d	-	+		d	-	-	d
dulcitol	-	-	-	-	-		-	-	-	-
glucose	d	-	+	-	+		+	+	d	d
inositol	-	-	-	-	-		-	-	-	-
lactose	-	-	-	-	-		-	-	-	-
maltose	-	-	-	-	+		+	+	d	+
mannitol	-	-	-	-	d		+	+	d	d
melezitose	-	-	-	-	-		+	-	d	d
melibiose	-	-	-	-	-		-	-	-	d
alpha methyl glucoside	-	-	-	-	-		d	+	d	d
raffinose	-	-	-	-	-		-	-	-	d
salicin	-	-	-	-	-		+	-	-	-
sucrose	-	-	-	-	d		+	d	d	+
xylose	-	-	-	-	-		-	-	-	+
use as sole carbon source										
adonitol	d	-	-	d	-	-	-	+	-	-
alanine	d	+	+	+	-	-	+	+	-	d
L+ arabinose	d	+	-	+	-	-	-	d	-	d
arginine	d	+	-	d	d	-	-	+	-	d
betaine	d	-	-	d	-	-	-	+	-	-
butanol	d	d	d	d	-	-	-	d	-	-
cellobiose	-	d	+	-	d	+	d	+	-	+
cystine	-	-	-	d	d	-	-	-	-	d

Table 5. (Continued)

Character	Group Designation									
	11	12	13	14	15	16	17	18	19	20
dulcitol	-	-	-	-	-	-	-	d	-	-
D fructose	-	d	+	+	d	d	+	d	+	+
D galactose	-	d	+	+	+	+	+	+	+	d
D glucose	-	d	+	+	+	+	+	+	+	+
glycerol	d	d	d	+	d	d	+	d	+	d
glycogen	-	d	d	+	+	+	d	-	+	-
inositol	-	-	-	-	-	-	d	-	+	d
inulin	-	-	-	d	d	-	-	-	-	-
lactose	d	+	d	-	d	+	+	d	+	+
lysine	-	d	-	+	d	+	-	-	-	d
malonic acid	-	-	-	-	d	+	-	-	-	d
maltose	+	+	+	+	+	+	+	+	+	+
mannitol	+	+	d	-	d	-	+	+	+	+
mannose	-	+	d	d	+	+	+	+	+	+
melezitose	d	+	-	+	d	-	+	d	+	+
melibiose	d	+	d	d	d	+	-	+	-	d
octanoic acid	d	d		-	-	-	-	+	-	d
ornithine	d	+	-	-	d	-	d	+	d	d
proline	-	-	d	-	+	+	+	+	+	d
propanol	-	-	-	-	d	+	-	d	-	d
rhamnose	+	d	+	+	d	-	d	+	+	+
ribose	+	+	-	-	d	+	+	+	-	+
salicin	d	+	+	-	d	d	d	d	d	+
salicylic acid	-	-	-	-	-	-	-	-	-	-
sodium acetate	d	+	d	+	-	-	d	+	d	d
sodium aspartate	+	+	d	+	d	-	d	+	-	+
sodium benzoate	-	-	-	d	-	-	-	-	-	-
sodium butyrate	d	-	-	-	-	-	d	d	-	-
sodium citrate	d	d	d	d	-	d	d	d	d	+
sodium formate	-	-	d	+	-	d	-	-	-	d
sodium glutamate	-	d	+	+	d	+	d	d	d	+
sodium lactate	+	+	d	+	d	+	d	+	+	d
sodium oxalate	-	-	-	-	-	-	-	-	-	-
sodium pyruvate	d	+	+	+		+	+	+	-	+
sodium succinate	d	+	d	-	d	-	d	+	d	+
serine	-	+	d	-	d	-	-	d	-	-
sorbitol	-	-	-	d	-	-	d	-	-	d
sucrose	+	+	+	-	d	d	d	d	+	+
trehalose	d	+	d	d	d	d	d	+	+	+
tryptophan	-	+	-	-	d	-	d	d	-	-
uracil	-	-	-	-	-	-	-	-	-	-
xylose	+	+	+	-	d	+	d	-	-	d

[a]- = less than 10 percent positive.

[b]d = 10 to 89 percent positive.

[c]+ = 90 to 100 percent positive.

[d]For the Gram stain only, - = Gram negative, var = Gram variable and + = Gram positive.

Table 6. Characteristics of groups 21 to 29 from the Center Hill Reservoir OTUs

Character	Group Designation									
	21	22	23	24	25	26	27	28a	28b	29
Colonial Morphology										
elevation										
peaked	–[a]	–	–	–	–	–	–	–	d[b]	–
flat	–	–	–	–	–	–	–	–	–	–
lumpy	–	–	–	–	–	–	–	–	–	–
raised	+[c]	d	+	+	+	+	+	+	d	+
umbonate	–	d	–	–	–	–	–	–	–	–
edge										
crenate	–	d	d	–	–	–	–	–	–	–
irregular	–	d	d	–	d	+	–	–	–	d
smooth	+	d	–	+	d	–	+	+	+	d
surface										
matt	–	–	–	–	–	d	–	–	–	–
shiny	+	d	d	+	–	–	–	–	–	–
waxy	–	d	d	–	+	d	+	+	+	+
transparency										
opaque	–	d	–	–	d	d	+	+	+	+
translucent	d	d	d	d	d	d	–	–	–	–
transparent	d	–	d	d	–	–	–	–	–	–
color										
white	–	–	d	+	d	d	+	–	d	–
off-white	–	d	d	+	–	d	–	–	–	–
light yellow	–	d	–	–	–	–	–	–	–	–
yellow	d	d	–	–	–	–	–	–	–	d
orange	d	–	–	–	–	–	–	+	–	d
pink	d	–	–	–	d	–	–	–	d	–
red	–	–	–	–	–	–	–	–	–	–
purple	–	–	–	–	–	–	–	–	–	–
diffusible pigment										
fluorescent pigment	–	–	d	–	–	–	–	–	–	–
Micromorphology										
length to width ratio										
coccoid	–	–	–	–	–	–	–	–	–	–
1.5-3:1	d	d	–	d	–	–	d	–	–	+
3-6:1	+	+	+	+	+	+	+	+	–	+
7-15:1	d	–	–	–	+	+	+	–	–	–
15:1	–	d	–	–	–	–	–	–	+	–
cell characteristics										
branching	–	–	–	–	–	–	–	–	–	–
round ends	+	+	+	+	+	+	+	+	+	+
tapered ends	–	–	–	–	–	–	–	–	–	–
stalked	–	–	–	–	–	–	–	–	–	–
curved	–	–	–	–	–	–	d	–	–	–
pleomorphic	–	–	–	–	–	–	–	–	–	+
fragmenting	–	–	–	–	–	–	–	–	–	–

Table 6. (Continued)

Character	Group Designation									
	21	22	23	24	25	26	27	28a	28b	29
chaining	–	–	–	–	–	d	–	d	–	–
rosette formation	–	–	–	–	d	–	–	–	–	–
motility										
gliding	–	–	–	–	–	–	–	–	–	–
wet mount	d	+	d	+	+	d	–	–	–	–
Staining Reactions										
Gram stain[d]	–	–	–	–	+	+	+	var	var	var
acid fast	–	–	–	–	–	–	–	d	–	–
granulation	–	–	d	–	–	d	+	–	–	d
Physiology										
salt tolerance										
2% NaCl	+	+	+	d	d	d	+	+	+	+
5% NaCl	d	d	–	d	d	d	+	–	–	–
7.5% NaCl	–	–	–	d	–	–	d	–	–	–
10% NaCl	–	–	–	–	–	–	d	–	–	–
12.5% NaCl	–	–	–	–	–	–	–	–	–	–
initiation of growth at pH										
pH 5	–	+	–	–	–	–	–	–	–	d
pH 6	+	+	+	+	d	+	+	+	+	+
pH 9	+	+	+	+	+	+	+	+	+	+
growth at temperature										
4°C	d	d	+	d	–	d	–	–	–	–
15°C	+	+	+	d	+	+	+	+	+	d
37°C	–	+	d	d	+	d	–	d	–	–
42°C	–	–	–	–	+	d	–	–	–	–
resistance to dyes and other inhibitors										
brilliant green	d	+	+	+	d	+	+	+	+	d
bismuth citrate	d	+	–	+	–	–	+	d	–	–
crystal violet	d	+	+	+	d	+	+	+	+	d
malachite green	d	+	+	+	d	+	+	+	+	d
methylene blue	d	+	+	+	+	+	+	+	+	+
potassium tellurite	–	–	–	–	–	–	–	d	–	–
pyronin B	d	+	+	+	+	+	+	+	+	+
sodium azide	–	–	–	–	–	–	–	–	–	–
Biochemistry										
acetoin production	–	–	d	–	–	d	d	–	d	–
arginine dehydrolase	d	–	+	–	–	d	d	–	–	–
catalase	d	+	+	+	+	+	+	d	+	+
indol production	–	–	d	–	–	–	–	–	–	–
levan production	–	d	–	–	–	–	–	d	–	–
lysine decarboxylase	–	d	d	–	–	–	–	–	–	–
methyl red reaction	–	–	d	–	–	d	–	–	d	–
nitrate reduction	d	d	+	d	d	+	d	+	d	–
nitrite reduction	d	d	–	d	–	–	d	d	–	–

Table 6. (Continued)

Character	Group Designation									
	21	22	23	24	25	26	27	28a	28b	29
ornithine decarboxylase	-	-	d	-	-	-	-	-	-	-
oxidase	d	d	d	d	-	d	d	-	+	d
glucose metabolism										
alkaline reaction	-	d	-	-	d	-	-	-	d	-
fermentative	d	d	d	-	-	+	d	d	-	d
oxidative	d	d	-	+	d	-	d	-	d	-
degradation of										
agar	-	-	-	-	-	-	d	-	d	-
casein	d	+	d	-	+	d	-	-	d	-
DNA	d	d	d	d	d	d	+	-	d	+
egg yolk	d	d	d	-	-	-	-	d	-	-
hypoxanthine	d	-	d	d	-	d	d	-	d	-
starch	d	-	d	-	-	d	-	-	-	-
Tween 20	d	d	+	+	d	d	+	+	-	-
Tween 40	d	d	+	+	-	d	+	+	d	+
Tween 60	d	d	+	+	-	d	+	d	d	+
Tween 80	d	d	+	d	d	d	-	+	d	+
tyrosine	d	+	d	+	-	d	-	-	-	-
urea	-	-	d	-	-	d	-	-	-	-
acid production from										
adonitol	d	-	-	-	-	-	d	-	-	-
arabinose	+	+	d	-	-	-	+	-	-	-
cellobiose	+	+	-	-	-	-	d	-	-	-
dextrin	d	d	+	+	-	d	-	-	-	-
dulcitol	-	-	-	-	-	-	-	-	-	-
glucose	+	+	d	+	-	d	+	-	-	-
inositol	d	d	-	-	-	-	-	-	-	-
lactose	d	d	-	-	-	-	-	-	-	-
maltose	+	d	+	d	-	d	+	-	-	-
mannitol	d	d	+	-	-	-	+	-	-	-
melezitose	-	-	-	-	-	-	-	-	-	-
melibiose	+	-	-	-	-	-	-	-	-	-
alpha methyl glucoside	d	-	-	-	-	-	-	-	-	-
raffinose	+	-	-	-	-	-	-	-	-	-
salicin	d	d	d	-	-	-	-	-	-	-
sucrose	+	d	d	-	-	-	+	-	-	-
xylose	+	d	-	-	-	-	+	-	-	-
use as sole carbon source										
adonitol	-	-	-	-	+	d	-	+	+	d
alanine	d	d	d	d	-	-	-	+	-	d
L+ arabinose	d	-	d	d	-	-	d	d	+	+
arginine	-	-	d	-	+	-	-	+	+	-
betaine	-	d	d	d	+	d	-	+	+	-
butanol	-	-	d	+	-	-	-	+	+	-
cellobiose	d	d	d	-	-	-	+	+	+	+
cystine	-	-	-	-	-	-	-	-	+	-

Table 6. (Continued)

Character	Group Designation									
	21	22	23	24	25	26	27	28a	28b	29
dulcitol	-	-	-	d	+	d	-	+	+	d
D fructose	+	d	+	+	+	+	-	+	d	+
D galactose	+	d	+	+	-	d	d	+	+	+
D glucose	d	d	+	+	+	d	d	+	+	+
glycerol	d	d	+	+	+	+	d	+	+	d
glycogen	d	d	d	d	-	d	d	+	+	+
inositol	+	d	-	d	+	d	-	d	+	d
inulin	d	d	-	-	+	-	d	d	d	d
lactose	+	d	-	-	-	-	d	d	+	+
lysine	-	d	d	-	d	-	d	+	+	+
malonic acid	-	-	-	-	-	-	-	+	+	-
maltose	d	d	+	-	+	d	+	+	+	+
mannitol	d	d	+	d	d	d	+	+	+	+
mannose	+	d	d	+	d	d	+	+	+	-
melezitose	d	-	d	-	+	d	-	-	+	+
melibiose	+	-	-	-	-	-	-	d	+	+
octanoic acid	-	d	-	-	-	-	d	-	-	-
ornithine	-	-	d	-	+	d	d	d	+	d
proline	d	d	+	d	+	d	+	+	d	-
propanol	-	-	-	d	+	-	-	+	+	+
rhamnose	d	d	d	+	d	-	d	d	+	+
ribose	d	d	d	-	+	+	+	+	+	+
salicin	d	d	d	-	+	d	+	-	+	d
salicylic acid	-	-	-	-	-	-	-	-	-	-
sodium acetate	-	-	d	d	+	d	-	+	+	d
sodium aspartate	d	d	+	+	+	d	+	+	+	-
sodium benzoate	-	-	-	-	-	-	-	-	d	-
sodium butyrate	-	-	-	-	d	-	-	-	-	-
sodium citrate	d	d	+	d	+	+	-	+	d	d
sodium formate	-	-	-	-	-	-	-	d	d	-
sodium glutamate	d	d	+	+	+	d	d	+	+	+
sodium lactate	d	d	d	-	+	d	+	+	+	+
sodium oxalate	-	-	-	-	-	-	-	d	-	-
sodium pyruvate	+	d	+	-	+	d	+	+	+	d
sodium succinate	d	d	d	+	+	d	d	+	+	+
serine	-	d	d	-	+	d	+	-	-	-
sorbitol	-	d	-	+	+	d	d	+	+	d
sucrose	d	d	-	-	d	d	+	-	d	-
trehalose	d	d	d	-	d	d	+	+	d	d
tryptophan	-	-	-	-	-	-	-	d	+	-
uracil	-	-	-	-	-	-	-	-	d	-
xylose	+	d	d	-	-	d	+	+	+	+

[a] - = less than 10 percent positive.

[b]d = 10 to 89 percent positive.

[c]+ = 90 to 100 percent positive.

[d]For the Gram stain only, - = Gram negative, var = Gram variable and + = Gram positive.

Table 7. Characteristics of groups 30 to 39 from the Center Hill Reservoir OTUs

Character	Group Designation									
	30	31	32	33	34	35	36	37	38	39
Colonial Morphology										
elevation										
peaked	–[a]	–	–	–	–	d[b]	d	–	+[c]	–
flat	–	–	d	–	–	–	–	–	–	d
lumpy	–	–	–	–	–	–	d	–	–	d
raised	+	+	d	+	+	d	d	+	–	d
umbonate	–	–	–	–	–	–	–	–	–	d
edge										
crenate	–	–	–	–	–	–	–	–	d	d
irregular	–	–	–	–	–	–	–	–	–	d
smooth	+	+	+	+	+	+	+	+	d	–
surface										
matt	–	–	–	–	–	–	–	–	–	–
shiny	d	–	+	+	+	–	–	–	+	+
waxy	d	+	–	–	–	+	+	+	–	–
transparency										
opaque	+	d	d	d	d	+	d	+	–	–
translucent	–	d	d	–	d	–	d	–	+	d
transparent	–	–	–	d	–	–	–	–	–	d
color										
white	d	–	d	d	–	–	–	–	–	–
off-white	–	d	d	d	d	–	–	–	d	+
light yellow	–	–	–	–	–	–	–	–	d	–
yellow	–	–	d	–	–	–	–	–	–	–
orange	d	d	–	–	d	+	–	+	–	–
pink	–	–	–	–	–	–	d	–	–	–
red	–	–	–	–	–	–	d	–	–	–
purple	–	–	–	–	–	–	–	–	–	–
diffusible pigment										
fluorescent pigment	–	–	–	–	–	–	–	–	–	d
Micromorphology										
length to width ratio										
coccoid	d	–	–	–	–	–	–	–	–	–
1.5-3:1	d	+	d	+	d	–	d	d	–	d
3-6:1	d	+	+	+	+	–	+	+	d	+
7-15:1	d	–	–	–	d	d	–	+	d	d
15:1	–	–	–	–	–	+	–	d	d	–
cell characteristics										
branching	–	–	–	d	d	+	–	–	–	–
round ends	+	+	+	+	+	+	+	+	+	+
tapered ends	–	+	–	–	–	d	–	–	–	–
stalked	–	+	–	–	–	–	–	–	–	–
curved	–	–	d	d	–	d	–	–	–	d
pleomorphic	d	+	–	–	–	d	d	–	–	–
fragmenting	–	–	–	–	–	d	–	–	–	–

Table 7. (Continued)

Character	Group Designation									
	30	31	32	33	34	35	36	37	38	39
chaining	-	-	-	-	-	-	-	-	-	-
rosette formation	-	-	d	-	-	-	-	-	-	-
motility										
gliding	-	-	-	-	-	-	-	-	d	-
wet mount	d	-	d	-	-	-	+	+	d	+
Staining Reactions										
Gram stain[d]	var	+	var	var	+	var	-	+	-	-
acid fast	-	-	-	-	-	-	-	-	-	-
granulation	-	-	-	-	-	-	+	-	-	-
Physiology										
salt tolerance										
2% NaCl	d	+	+	d	+	+	-	+	d	+
5% NaCl	d	-	+	d	+	-	-	+	d	d
7.5% NaCl	-	-	d	-	d	-	-	+	-	-
10% NaCl	-	-	d	-	d	-	-	-	-	-
12.5% NaCl	-	-	d	-	-	-	-	-	-	-
initiation of growth at pH										
pH 5	d	-	+	-	-	+	+	-	-	-
pH 6	+	+	+	+	+	+	+	+	+	+
pH 9	+	+	+	+	+	+	+	+	+	+
growth at temperature										
4°C	d	+	d	d	d	-	-	d	+	+
15°C	+	+	+	+	+	+	+	+	+	+
37°C	d	-	+	d	-	+	d	d	+	d
42°C	d	-	-	-	-	+	d	-	-	-
resistance to dyes and other inhibitors										
brilliant green	+	+	+	+	+	d	+	+	+	+
bismuth citrate	+	+	+	-	+	d	d	+	-	d
crystal violet	d	+	+	+	+	d	+	+	+	+
malachite green	+	+	+	+	+	d	+	+	+	+
methylene blue	+	+	+	+	+	d	+	+	+	+
potassium tellurite	d	-	d	-	+	d	d	+	-	-
pyronin B	+	+	+	+	+	d	+	+	+	+
sodium azide	-	-	-	-	-	-	-	-	-	-
Biochemistry										
acetoin production	-	-	-	-	-	d	-	-	-	-
arginine dehydrolase	-	d	-	-	d	-	-	-	-	+
catalase	+	+	+	+	+	+	+	+	+	+
indol production	d	-	-	-	-	-	-	-	-	-
levan production	-	-	d	-	+	-	-	d	-	-
lysine production	-	-	-	-	-	-	d	-	-	-
methyl red reaction	-	-	-	-	-	-	-	-	-	-
nitrate reduction	+	+	d	d	-	d	d	d	+	d
nitrite reduction	d	+	d	d	-	d	d	-	d	d

Table 7. (Continued)

Character	Group Designation									
	30	31	32	33	34	35	36	37	38	39
ornithine decarboxylase	-	-	-	-	-	-	-	-	d	-
oxidase	d	d	d	-	d	-	d	d	-	+
glucose metabolism										
alkaline reaction	d	-	-	d	-	d	-	d	+	-
fermentative	-	+	d	d	-	d	d	-	-	d
oxidative	d	-	d	d	+	d	-	+	+	d
degradation of										
agar	-	-	-	-	-	d	-	-	-	-
casein	-	d	+	-	+	d	-	d	+	+
DNA	d	d	d	d	+	d	-	d	+	d
egg yolk	-	d	-	-	-	-	-	d	+	+
hypoxanthine	d	d	+	-	-	d	-	-	-	+
starch	-	+	+	d	+	+	d	-	d	-
Tween 20	d	d	d	d	+	-	-	d	+	d
Tween 40	d	-	d	+	+	d	-	d	d	d
Tween 60	d	-	d	d	+	d	-	+	d	d
Tween 80	d	-	d	d	+	d	-	d	d	d
tyrosine	d	+	+	d	+	d	-	+	d	+
urea	-	-	d	-	d	-	-	d	d	d
acid production from										
adonitol	-	-	-	-	-	-	-	-	-	d
arabinose	-	-	-	d	d	-	+	d	d	+
cellobiose	-	-	-	-	-	-	-	-	-	d
dextrin	-	-	-	-	-	-	-	-	d	-
dulcitol	-	-	-	-	-	-	-	-	-	-
glucose	-	-	d	d	+	-	d	+	d	+
inositol	-	-	d	-	-	-	-	-	-	d
lactose	-	d	-	-	+	-	-	-	d	-
maltose	-	d	-	-	d	-	-	-	-	d
mannitol	-	-	-	-	+	-	-	d	-	+
melezitose	-	-	-	-	+	-	-	-	-	-
melibiose	-	d	d	-	-	-	-	-	-	d
alpha methyl glucoside	-	-	-	-	-	-	-	-	-	-
raffinose	-	-	-	d	-	-	-	-	-	-
salicin	-	-	-	-	-	-	-	-	-	-
sucrose	-	-	-	-	d	-	-	+	-	d
xylose	-	-	-	d	d	-	+	d	-	+
use as sole carbon source										
adonitol	+	-	d	d	-	-	+	d	-	d
alanine	+	d	d	d	+	d	d	+	+	d
L+ arabinose	d	-	d	d	+	+	+	+	+	d
arginine	+	-	d	-	d	-	d	+	+	+
betaine	+	d	d	-	d	-	d	+	-	d
butanol	+	-	d	-	+	-	d	+	-	d
cellobiose	d	+	d	+	+	+	d	d	+	-
cystine	d	-	d	-	-	-	d	-	-	-

Table 7. (Continued)

Character	Group Designation									
	30	31	32	33	34	35	36	37	38	39
dulcitol	d	-	d	d	-	-	+	+	d	d
D fructose	+	+	+	+	+	+	d	+	-	+
D galactose	+	+	+	+	+	+	+	+	d	+
D glucose	+	+	+	+	+	+	+	+	+	+
glycerol	+	+	+	+	+	-	+	+	d	+
glycogen	+	+	+	d	+	+	d	+	d	d
inositol	+	-	d	d	+	-	-	-	+	d
inulin	d	-	d	d	d	-	d	+	-	-
lactose	+	+	+	d	+	+	d	+	+	+
lysine	d	d	d	d	+	+	d	+	+	+
malonic acid	d	-	d	d	+	-	d	+	-	d
maltose	+	+	+	+	+	d	+	+	+	-
mannitol	d	+	+	d	+	d	+	+	+	+
mannose	d	+	+	+	+	d	-	+	-	+
melezitose	+	+	+	-	+	-	d	+	+	-
melibiose	d	+	+	+	+	d	+	+	+	d
octanoic acid	-	-	d	d	-	-	-	+	-	+
ornithine	d	d	d	d	d	-	d	+	d	d
proline	+	+	+	d	+	d	+	+	+	+
propanol	d	d	d	-	-	d	d	+	+	d
rhamnose	d	+	+	d	d	-	d	+	+	d
ribose	d	+	+	d	-	d	+	+	+	+
salicin	d	-	d	d	d	d	-	-	+	-
salicylic acid	-	-	-	-	-	-	-	-	-	-
sodium acetate	+	-	d	d	+	d	d	d	-	d
sodium aspartate	d	d	d	+	+	-	+	+	+	+
sodium benzoate	d	-	d	-	d	-	+	+	-	d
sodium butyrate	+	-	d	d	-	-	+	+	-	d
sodium citrate	+	+	d	d	+	d	+	-	d	+
sodium formate	d	-	d	-	-	-	d	+	+	d
sodium glutamate	+	+	+	d	+	+	+	+	+	+
sodium lactate	+	+	+	-	+	d	+	+	d	-
sodium oxalate	d	-	d	-	-	-	d	+	-	d
sodium pyruvate	+	+	+	-	+	d	+	+	+	+
sodium succinate	+	+	d	+	+	+	+	+	+	d
serine	d	-	d	d	d	-		d	+	d
sorbitol	-	-	+	d	+	-	d	+	-	d
sucrose	+	-	d	+	+	d	d	d	-	d
trehalose	d	-	d	+	+	-	+	d	+	d
tryptophan	d	-	d	-	-	d	d	+	-	-
uracil	d	-	-	d	d	-	-	+	-	d
xylose	d	+	+	+	+	+	+	+	+	d

[a] - = less than 10 percent positive.

[b]d = 10 to 89 percent positive.

[c]+ = 90 to 100 percent positive.

[d]For the Gram stain only, - = Gram negative, var = Gram variable and + = Gram positive.

Table 8. Characteristics of groups 40 and 41 from the Center Hill Reservoir OTUs

Character	Group Designation 40	41
Colonial Morphology		
elevation		
peaked	-[a]	-
flat	-	-
lumpy	d[b]	-
raised	d	-
umbonate	-	+[c]
edge		
crenate	d	d
irregular	d	-
smooth	d	d
surface		
matt	-	-
shiny	+	d
waxy	-	d
transparency		
opaque	d	+
translucent	d	-
transparent	-	-
color		
white	d	+
off-white	d	-
light yellow	-	-
yellow	-	-
orange	-	-
pink	-	-
red	-	-
purple	-	-
diffusible pigment		
fluorescent pigment	d	-
Micromorphology		
length to width ratio		
coccoid	-	-
1.5-3:1	d	d
3-6:1	+	d
7-15:1	d	+
15:1	-	-
cell characteristics		
branching	-	-
round ends	+	+
tapered ends	d	-
stalked	-	-
curved	-	-
pleomorphic	-	-
fragmenting	-	-

Table 8. (Continued)

Character	Group Designation 40	41
chaining	d	-
rosette formation	-	-
motility		
gliding	-	-
wet mount	+	+
Staining Reactions		
Gram stain[d]	-	-
acid fast	-	-
granulation	d	-
Physiology		
salt tolerance		
2% NaCl	d	+
5% NaCl	d	+
7.5% NaCl	d	+
10% NaCl	-	-
12.5% NaCl	-	-
initiation of growth at pH		
pH 5	+	-
pH 6	+	-
pH 9	+	+
growth at temperature		
4°C	+	d
15°C	+	+
37°C	d	d
42°C	d	d
resistance to dues and other inhibitors		
brilliant green	+	+
bismuth citrate	+	+
crystal violet	+	+
malachite green	+	+
methylene blue	+	+
potassium tellurite	d	-
pyronin B	+	+
sodium azide	-	-
Biochemistry		
acetoin production	-	-
arginine dehydrolase	+	d
catalase	+	+
indol production	-	-
levan production	-	-
lysine decarboxylase	-	-
methyl red reaction	-	-
nitrate reduction	d	-
nitrite reduction	d	-

Table 8. (Continued)

Character	Group Designation 40	41
ornithine decarboxylase	d	-
oxidase	d	-
glucose metabolism		
alkaline reaction	-	-
fermentative reaction	d	+
oxidative	d	-
degradation of		
agar	-	d
casein	d	+
DNA	d	+
egg yolk	d	-
hypoxanthine	d	-
starch	-	-
Tween 20	d	+
Tween 40	d	+
Tween 60	d	+
Tween 80	d	d
tyrosine	+	+
urea	d	-
acid produciton from		
adonitol	-	-
arabinose	+	+
cellobiose	d	+
dextrin	d	-
dulcitol	-	-
glucose	+	+
inositol	d	+
lactose	d	d
maltose	d	+
mannitol	d	+
melezitose	-	-
melibiose	+	d
alpha methyl glucoside	-	-
raffinose	-	-
salicin	-	-
sucrose	+	d
xylose	+	+
use as sole carbon source		
adonitol	d	d
alanine	d	+
L+ arabinose	+	+
arginine	d	+
betaine	+	d
butanol	+	+
cellobiose	d	+
cystine	-	-

Table 8. (Continued)

Character	Group Designation 40	41
dulcitol	d	d
D fructose	+	+
D galactose	d	+
D glucose	+	+
glycerol	+	+
glycogen	d	-
inositol	d	+
inulin	d	-
lactose	d	d
lysine	+	-
malonic acid	-	+
maltose	d	+
mannitol	+	d
mannose	d	+
melezitose	d	-
melibiose	d	-
octanoic acid	+	d
ornithine	d	+
proline	+	+
propanol	d	+
rhamnose	-	-
ribose	+	+
salicin	d	-
salicylic acid	d	-
sodium acetate	d	-
sodium aspartate	d	+
sodium benzoate	d	-
sodium butyrate	-	-
sodium citrate	+	+
sodium formate	d	d
sodium glutamate	d	d
sodium lactate	d	+
sodium oxalate	d	d
sodium pyruvate	d	d
sodium succinate	+	-
serine	d	d
sorbitol	+	d
sucrose	+	d
trehalose	+	-
tryptophan	-	+
uracil	d	-
xylose	+	+

[a]- = less than 10 percent positive.

[b]d = 10 to 89 percent positive.

[c]+ = 90 to 100 percent positive.

[d]For the Gram stain only, - = Gram negative.

Isolation Media and Group Relationships

Distribution of OTUs as a Function of Their Isolation Medium

Only one group, group 2, contained OTUs isolated from all four isolation media. Seven groups, 17 percent of all groups, contained members from three isolation media. Eight groups contained members from only one medium. The remaining groups, having strains isolated from two media, accounted for 61 percent of the defined groups. Figure 4 summarizes the distribution of OTUs in phenetic groups by their isolation media. It is interesting to note that of the 18 groups containing an OTU isolated from YEPGA, only 5 of these groups contain an OTU isolated from PHA. In all of these five groups, group 2 included, only one OTU isolated from PHA is a member of each group. This suggests that taxa isolated from YEPGA and PHA are nearly mutually exclusive. If each unclustered strain represents a distinct taxonomic group, then a total of 100 taxa were recovered in this study. OTUs isolated from YEPGA, PHA and PCBA were recovered as single strains only 25 percent of the time. This relationship is demonstrated in Figure 5. Strains isolated from LWA were recovered as single strains 41 percent of the time. Of those groups containing an OTU isolated from YEPGA, 33 percent were made up exclusively of YEPGA isolates (Fig. 4A). For the other three media the frequency of exclusive groups was lower, i.e., 6, 18, and 9 percent for LWA, PHA, and PCBA isolates respectively. From Figure 4C it can be observed that in over 50 percent of groups containing an OTU taken from PHA there is also an OTU recovered from PCBA. The inverse relationship also is observed (Fig. 4D). Of those groups containing an OTU isolated from LWA, 56 and 50 percent contain

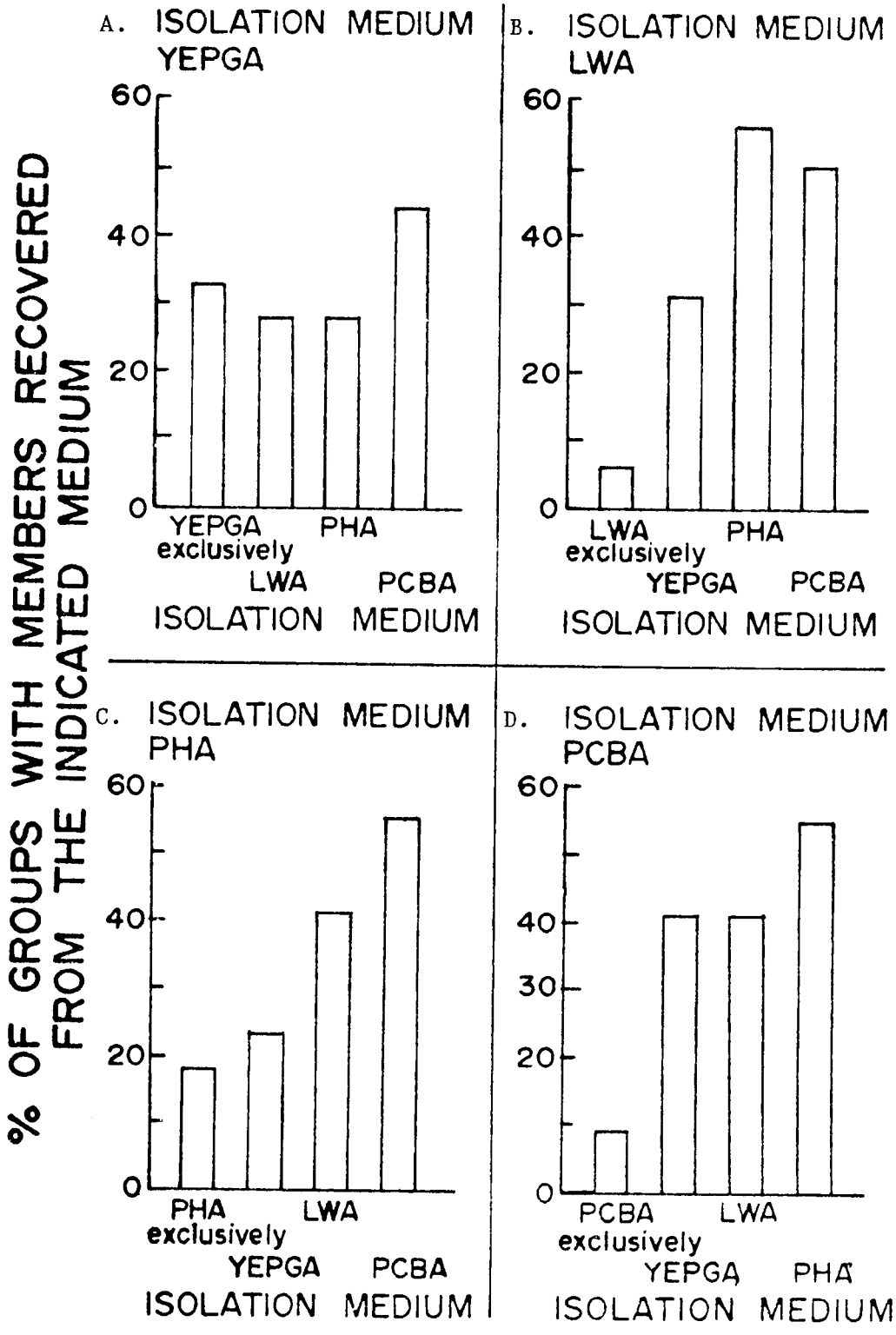


Figure 4. The distribution of OTUs from the four isolation media within defined groups.

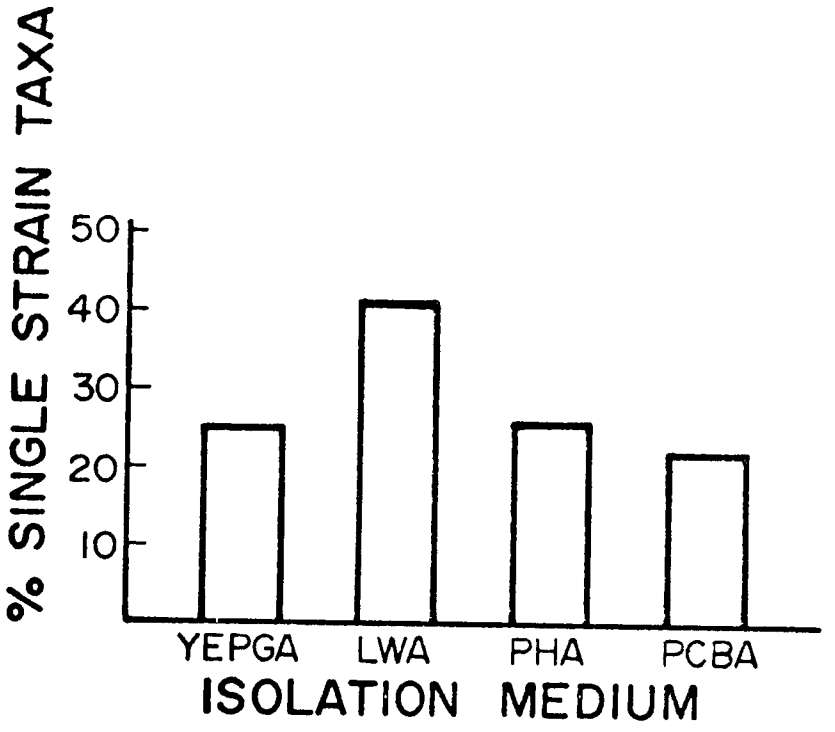


Figure 5. The recovery of single strain taxa as a function of OTU isolation medium.

an OTU from PHA and PCBA respectively (Fig. 4B). The inverse relationship, however, does not hold. This is due to the number of groups containing LWA, PHA and PCBA. While both PHA and PCBA isolates are contained in 22 groups each, LWA isolates are only found in 16 groups.

The number of OTUs taken from each isolation medium and the total number of taxa characterized, including single strains, from each medium is given in Table 9. It is interesting to note that the OTUs isolated from LWA had the greatest apparent species richness (number of taxa per total OTUs characterized).

Physiological Groupings of the Clustered OTUs

By using the degradative characters and the sole carbon characters, four large groups can be defined from those OTUs contained in groups (Tables 4 through 8). These are (i) groups 1 to 11 which average 4.6 carbon sources utilized, (ii) groups 12 to 20 which averages 15.8 carbon sources utilized, (iii) groups 21 to 24 which average 7.5, and (iv) groups 25 to 41 which average 21 carbon sources utilized. There was no significant difference between groupings i and ii when carbon source characters which are 90 to 100 percent positive ("+" on the tables) were compared. However, when carbon source characters which are 10 to 90 percent positive within a group were included, it was found that grouping iii averaged 28 carbon sources totally to partially utilized within a group while grouping i averaged 21 carbon sources. When partial and total positive degradative characters were examined, grouping i averaged 4.5 characters while grouping iii averaged 9 characters. With the exception of grouping iv,

Table 9. Total viable counts, OTU recovery and taxa defined from Center Hill Reservoir

	Isolation Medium			
	YEPGA	LWA	PHA	PCBA
Total viable count[a]	3,600	2,400	180	370
OTUs recovered	60	46	47	59
Cumulative OTUs	60	106	153	212
Taxa characterized per medium	33	35	34	35
Cumulative taxa	33	63	85	100

[a]Expressed as colony forming units per ml lake water.

groups 25 to 41, the groupings do not correspond to any observed higher order grouping as delineated by the simplified dendrogram (Fig. 3).

Examination of the Inverted Matrix

When the characters were examined using the simple matching coefficient in the inverted matrix, it was observed that 43 of the 50 carbon source characters were clustered in 9 groups at the 50 to 60 percent similarity level. Significant intragroup structure was observed in many of the groups. This intragroup structure was defined at or above the 70 percent similarity level. When examined using the S_J coefficient, similarity was lower but group structure and intragroup structure were largely maintained. Most groups could be characterized according to the chemical nature of the carbon sources. Groups fell into five broad classes: simple hexoses, disaccharides and polysaccharides, amino acids, sugar alcohols, and organic acids.

No significant correlation was seen between acid production characters and sole carbon source characters. With such a wide spectrum of OTUs a higher correlation was observed within acid production characters.

IV. DISCUSSION

In order to discuss the direct results of this investigation it is necessary to acknowledge certain limitations affecting the interpretation of the data. The use of numerical taxonomy to classify bacteria is limited by the number of OTUs examined, the number and appropriateness of characters tested, and the similarity coefficients and clustering techniques employed (100). However, bias introduced into the study through the use of numerical taxonomic techniques, most likely, is overshadowed by problems associated with the isolation, relative recovery, and culture of bacteria from their native aquatic environment. While the method of choice for isolation appears to be the dilution spread plate technique (18), there is no good choice for a plating medium capable of supporting the growth of all native aquatic bacteria (28). It is exceedingly unlikely that any isolation medium can provide optimum growth conditions for bacteria colonizing diverse habitats, or existing in various physiologically supressed or dormant states as described by Stevenson (103).

In this study it was assumed that those bacterial populations which are most fit, in terms of growth potential in the aquatic environment, and are best adapted to the isolation medium and are present in relatively high numbers will be recovered on the various isolation media in greatest frequency. In addition, it was assumed that aggregation was not a significant problem. Given the above concepts, when the number of OTU isolation media are increased, the number of observed taxa should also increase. This hypothesis was supported by our results

(Table 9). It can also be reasoned that "fit" taxa will be recovered on more than one isolation medium while dormant or stressed populations having restricted growth ranges will be recovered on fewer isolation media, hence, fewer new taxa per isolation medium. This hypothesis is also supported by the results of the investigation (Table 9).

No single taxon within the bacterial guild was found to be dominant in this investigation. The largest group, phenon 2 (Fig. 3), contained only 9 percent of the total isolates, while the second largest phenon contained only 5 percent. Bacterial diversity of the sample can by examined using the Shannon diversity index $\overline{H}$, where $\overline{H} = c/n\ (N \log_{10} N - \Delta n_i \log_{10} n_i)$. N is the total number of OTUs examined, n_i is the total number of individuals in the i^{th} group and c is a constant equal to 3.3219. $\overline{H}$ was found to be 3.21 indicating high diversity. This level of diversity is comparable to oligotrophic marine habitats. Martin and Bianchi (72), using more stringent definitions of taxa (S_{sm} coefficient defining groups at the 90 percent level), reported $\overline{H}$ values ranging from 3.6 to 4.0. Other investigators (59) have reported $\overline{H}$ values ranging from 1.0 to 4.0 in the Beaufort Sea again using stringent taxa definition (S_J coefficient at the 70 percent level). The use of multiple isolation media, ranging from low nutrient to high nutrient, resulted in increased $\overline{H}$ values in this study.

The goodness of fit of the classification reported was demonstrated by the minor group rearrangement after controlling for group formation due to negative matching. However, as reported in other investigations (3, 10, 62, 71, 95), unequivocal identification of all defined groups was impossible. Only 58 percent of the groups could be

identified and only 79 percent of these to the genus or species level. Rather than being a fault of the numerical technique, this problem is most likely due to the nature or definition of a bacterial species. Bacterial taxa can be considered to consist of a spectrum or series of integrating forms or phenotypes that gives rise to populations that cannot unequivocally be identified using the phenetically defined clusters (23, 42).

The bacterial populations recovered from each of the four media suggest that each medium selects for different populations (Figs. 4A-D and 5). YEPGA was found to be the most selective; 25 percent of the OTUs isolated from YEPGA were recovered as single strain taxa and 33 percent of the groups containing OTUs from YEPGA were exclusive to that medium. Over 50 percent of the taxa isolated from YEPGA were exclusive to that medium.

The degree of selectivity found in YEPGA was not observed among the other three media. However, their patterns of OTU isolation are by no means identical. All three media can be selective for oligotrophic bacteria. Although two media, PHA and PCBA, contain high concentrations of carbon, i.e., phenanthrene and polychlorinated biphenyls respectively, these compounds cannot be used for growth by many groups of bacteria. Plate counts (Table 9) would indicate that these media are somewhat toxic to many of the bacteria found in the water column. Even if an organism should be resistant to one of the toxicants, the only available carbon for growth would be contaminants in the medium. Thus in order to initiate growth an organism would have to be resistant to the toxicant and an oligotrophe, or be able to utilize the toxicant.

In this way PHA and PCBA could select for oligotrophic as well as degradative bacteria. This form of selection has been demonstrated and discussed by DiGeronimo et al. (31). Species richness was lower for PHA and PCBA when compared to LWA. PCBA was found to be the least selective medium in this study. OTUs isolated using PCBA exhibited the lowest percent single strain taxa and the lowest percent of groups recovered as exclusive PCBA isolates (Figs. 4D and 5). This finding is not surprising. Sayler at al. (88), Shiaris at al. (92) and Blakemore and Carey (11) have demonstrated that highly recalcitrant polychlorinated biphenyls appear to have only moderate to no biological effects on heterotrophic bacterial activity. The two media PHA and PCBA were found to select for similar taxa. This is to be expected in that the media differ only in the type of toxicant present. In addition the taxa selected were similar to those recovered from LWA.

If we assume that carbon source utilization represents a component of the fundamental heterotrophic role, it is possible to examine the hypothetical niche these organisms occupy. It is significant that nine definable groups of utilizable carbon sources were recovered in the inverted matrix despite the permissive nature of carbon source testing. This resulted in the elucidation of five major classes of carbon source utilization patterns within the major groups of carbon sources. Those results aid in defining generalized niches, in that organisms may specialize with respect to carbon source utilization within the generalized niche, yet there is sufficient similarity to result in definable but overlapping utilization patterns. If Hutchinson's (53) portrayal of the aquatic environment as a dynamic system is accepted, it follows

that potential niches for heterotrophic bacteria, as measured by available carbon sources, are also in a dynamic state. Consequently the physiological and biochemical potential of the individual defines the possible occupiable niche. If the niche is sufficiently stable over time, competition among co-existing populations would result in a narrowing of definable taxa. Conversely, if the potential niche structure is rapidly changing within a community, similar taxa, but genetically distinct, could occupy the same niche. Consequently, physical transport or survival may be more important than unique adaptations in the ability of an organism to occupy a given niche. These relationships are distorted by the artificiality of the laboratory environment; however, they do provide a conceptual framework in which to view the structure and function of the bacterial guild within the aquatic community.

PART III: NUMERICAL TAXONOMY OF BACTERIA FROM SAUCON CREEK

I. INTRODUCTION

Lipid analysis has been used in both ecological and taxonomic studies (13, 68). Lipids commonly examined include fatty acids and phospho lipids (13, 54, 68). Lipids make up a large proportion of the cellular membrane and, therefore, are compounds of fundamental importance to all cells. The basic function and chemical nature of the cellular membrane is similar for all bacterial cells (104). This allows for direct comparisons between highly diverse taxa using, in many cases, a single method (13, 68).

Fatty acids have been extensively studied. While all fatty acids have essentially the same chemical nature, they are an extremely diverse group of compounds. These properties have made fatty acid analysis an important factor in the chemotaxonomic examination of bacterial populations. A number of reviews have been published on the role of lipids in bacterial taxonomy (38, 68, 89). More recently lipid analysis has been applied to microbial ecological problems (12, 13, 27, 54, 55, 56, 65). With the aid of high resolution gas-liquid chromatography, investigators, in a recent study, used fatty acid analysis to estimate microbial community structure (13).

By using species as the unit of measure within a community, the elucidation of bacterial guild structure has been hampered by a number of factors: (i) recovery of populations; (ii) isolation, in those cases where pure culture characterization is attempted; (iii) characterization; and (iv) classification. An overriding factor, often overlooked, is the nature of the bacterial species. Cowen (23, 24, 25) proposed that

bacterial species vary in a spectrum-like manner. Gordon, largely in support of Cowen, proposed that a species consists of a population made up of old and new isolates from as many different environments as possible having a high degree of phenetic, biochemical and genetic similarity (42). The concept of a group of similar strains, a population, is central to this species concept. Thus, species designations are dependent on the concentration of similar strains over time and within different environments.

The bacterial populations present within a given community (bacterial guild) are dependent upon a number of factors. Principle factors include available water, mean and extreme temperatures, pH, Eh, available oxygen, osmolarity, available energy sources, nutrients, and activity or dormancy of the cells. In a constantly changing environment, such as most aquatic environments, the available roles within a community are constantly shifting. Therefore, unutilized or underutilized (unfilled or partially filled niches) become available. In a changing environment one may hypothesize that these unfilled or partially filled niches may be filled, more often than not, by bacteria which are present and capable of growth rather than the most competitively fit species. Hutchinson (53) proposed such mechanisms in discussing the high diversity found in planktonic communities.

The results of numerical taxonomic study of the definition and characterization of phenetic groups of a bacterial guild from a single community may be taken as measures of the fundamental niche of each group. In a previous study of an aquatic bacterial guild, high diversity was noted (Part II). Evidence for a significant degree of

heterogeneity within defined groups also was noted. This variation was most noticeable for specific biochemical characters such as the oxidase, catalase, nitrate and nitrite reductase and the oxidation or fermentation of glucose. Gram stain characters also exhibited a significant variation within certain groups. This intragroup character variation has been noted in other studies where environmental isolates have been examined (3, 62, 71). Identification in these studies has been a problem. In regards to identification, variation of a number of key characters has proven to be a major stumbling block.

The general purpose of this study was to conduct a comparative investigation of the nature of the bacterial guild of an aquatic community using both a phenetic and a chemotaxonomic approach. Specific goals of this study were threefold: (i) to test and establish the suitability of fatty acid methyl ester (FAME) analysis coupled with numerical taxonomic techniques as a method to classify and characterize aquatic bacteria; (ii) to compare classifications obtained using phenetic and chemotaxonomic data; and (iii) to relate similarities and differences between the two classification systems to taxonomic species, using Gordon's definition, and to niche structure, using the phenetic estimates of the fundamental niches.

II. MATERIALS AND METHODS

Sample Site Characterization and OTU Isolation

Sample Site Location and Characterization

Sediment samples were collected from Saucon Creek, a shallow, freshwater stream at Bethlehem, Pennsylvania, which has previously been described (83, 87, 88). Sample site locations for the samples taken on June 19, 1979 are shown in Figure 6. Dissolved oxygen, temperature and conductivity were measured at the sediment water interface at the time of sampling. Other chemical measurements of field samples, as well as estimates of bacterial population densities, biomass and activity, have been previously reported for a sampling regime ranging from July 1978 to September 1979 (83, 87, 88).

The physical, chemical, biochemical, and biological data were logarithmically transformed and subjected to computerized statistical analysis. Discriminant analysis was performed using the SPSS program package. Multivariate analysis of variance and multiple range analysis was performed using programs within the SAS program package. Listwise deletion of missing variables was used in both the discriminant analysis and the multivariate analysis of variance.

Operational Taxonomic Unit Isolation and Maintenance

Isolated colonies were acquired by using the spread plate technique. Sediment dilutions were inoculated onto phenanthrene-basal salts agar (PHA) (88). After 4 weeks incubation at 20°C in the dark, isolated colonies, chosen at random, were streaked repeatedly on

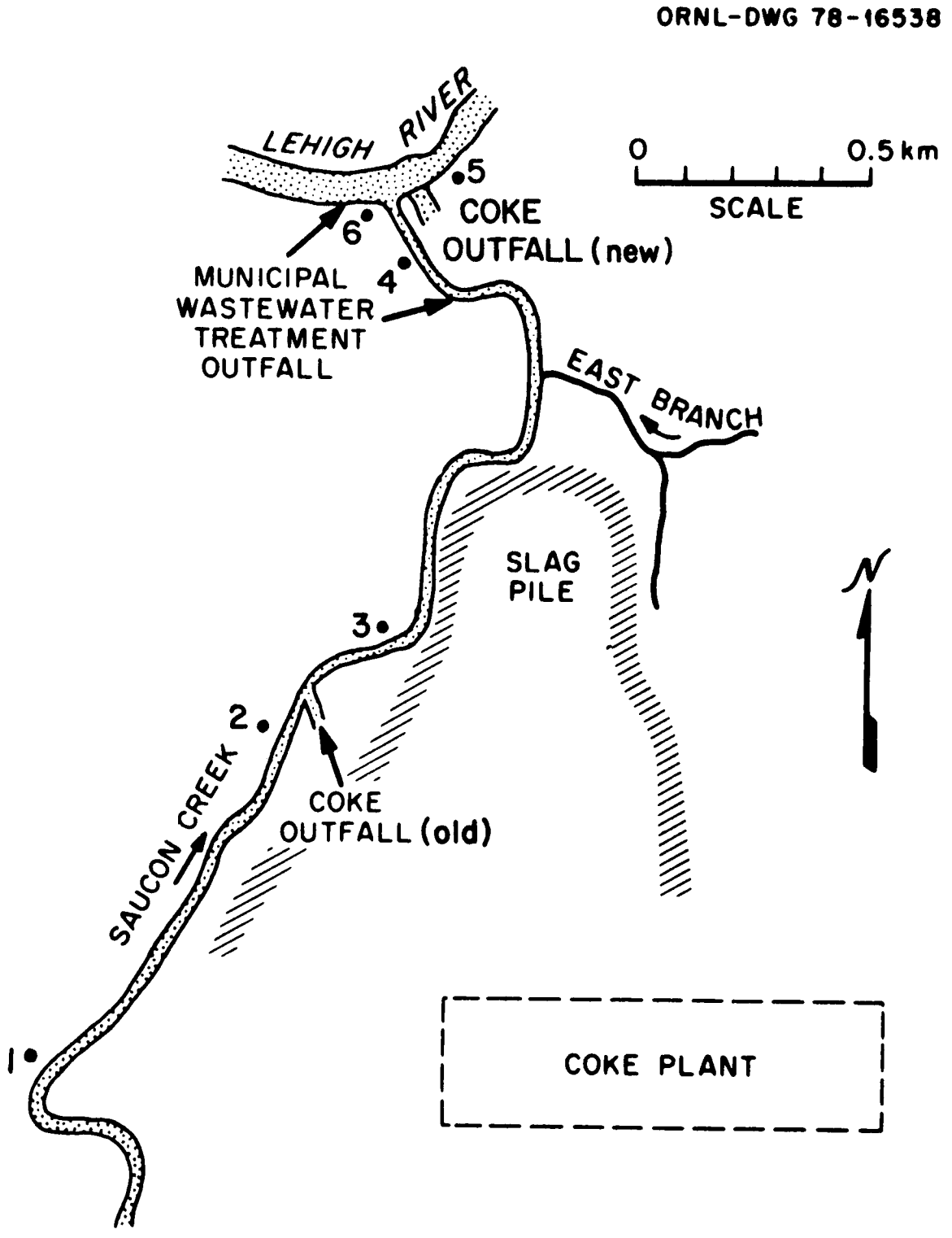


Figure 6. Sample sites on Saucon Creek, Pennsylvania.

a yeast extract-phenanthrene agar medium (YEPHA) until pure cultures were obtained. The YEPHA had a pH of 7.2 and contained 0.05 percent (w/v) yeast extract, 0.05 percent (w/v) phenanthrene and 1.8 percent (w/v) purified agar in distilled water. Culture purity was assessed using colonial morphology, Gram stain reaction and phase contrast microscopy. The isolated strains were the operational taxonomic units (OTUs) in this investigation. A total of 60 OTUs were examined. The OTUs were maintained on YEPHA slants. Fresh slants were inoculated every 6 to 8 weeks.

Phenetic Classification

OTU Characterization

The OTUs were examined for a total of 84 phenetic characters. The characters used are listed in Table 10. The methods used were identical to those reported previously (Part II). The following exceptions should be noted. The maximun incubation time used was 2 weeks. Where a basal medium of yeast extract-peptone-glucose agar was used in the earlier study, a basal medium consisting of 0.05 percent (w/v) yeast extract and 1.8 percent purified agar was substituted. Where applicable, e.g., salt tolerance characters and degradation characters, the modified basal medium was adjusted to pH 7.2.

Phenetic Data Analysis

The data were coded into a binary format using non-additive coding where applicable (100). As in the previous study, noncomparable results were coded as "9". A positive result was coded as "1" and a negative result was coded as "0".

Table 10. Percent positive frequency for phenetic characters used in the computer analysis of the Saucon Creek OTUs

Character	Percent	Character	Percent
Colonial Morphology		resistance to inhibitors	
elevation		bismuth citrate	10
flat	5	Aroclor 1254	85
peaked	7	growth at temperature	
raised	88	4°C	35
edge		15°C	95
crenate	21	37°C	38
smooth	79	42°C	8
surface		survival at temperature	
shiny	84	42°C	42
waxy	16	Biochemistry	
transparency		catalase	94
opaque	31	nitrate reduction	60
translucent	39	nitrite reduction	8
transparent	30	oxidase	25
color		glucose metabolism	
off-white	70	alkaline reaction	13
yellow	29	fermentative	62
pink	1	oxidative	26
Micromorphology		no reaction	12
length to width ratio		degradation of	
coccoid	8	casein	35
1.5-2:1	30	DNA	33
2-4:1	77	hypoxanthine	17
5-7:1	73	starch	24
7-12:1	22	Tween 80	56
filaments	3	tyrosine	64
cell characteristics		utilization as a sole carbon source	
branching	3	adonitol	30
chaining	7	alanine	68
curved cell	32	arabinose	53
granulation	10	arginine	57
pleomophic	12	butanol	63
swelling	8	cellobiose	63
motility		galactose	73
wet mount	17	glucose	78
Staining Reactions		glycerol	88
Gram positive	38	glycogen	23
Gram variable	5	inositol	48
Gram negative	57	lactose	45
Physiology		leucine	48
salt tolerance		lysine	43
2% NaCl	81	mannitol	63
5% NaCl	53		

Table 10. (Continued)

Character	Percent
melezitose	50
melibiose	51
propanol	63
ribose	75
salicin	60
sodium acetate	80
sodium aspartate	82
sodium butyrate	17
sodium citrate	77
sodium glutamate	78
sodium lactate	72
sodium pyruvate	83
sodium succinate	81
serine	59
sucrose	75
tryptophan	33
xylose	65

The coded data were examined using the simple matching coefficient (S_{sm}) (101) and the Jaccard coefficient (S_J) (97). The S_J coefficient was used primarily to control for negative matching. The resulting similarity matrices were clustered using an unweighted average linkage method (UPGMA) (100).

FAME Classification

Fatty Acid Extraction and Characterization

OTUs were streaked onto a plating medium having a pH of 7.2 and containing 0.15 percent (w/v) Trypticase (Difco), 0.05 percent (w/v) Phytone (Difco), 0.05 percent (w/v) sodium chloride and 1.8 percent (w/v) purified agar. The strains were incubated at 28°C for 36 hours. Eight of the OTUs did not grow or grew poorly within the incubation time. These strains were not included in the analysis.

Cells were carefully scraped off the plates into a test tube containing 5 ml of 5 percent sodium hydroxide in 50 percent aqueous methanol. Saponification and methylation were carried out using method B of Moss et al. (79). FAME extracts were stored at 4°C under nitrogen. Six OTUs were run in duplicate in order to assess reproducibility. Duplicate OTUs were not extracted and analyzed at the same time.

The extracts containing the FAME were assayed using a Perkin Elmer 3920 gas-liquid chromatograph equipped with a flame ionization detector. An electronic integrator was used to determine peak area. A 6 foot glass column with a 2 mm inner diameter, packed with 3 percent OV101 on Chromosorb Q (Supelco), was used for the analysis. The injector and detector were maintained at 300°C. Following injection

the column temperature was held at 150°C for 2 minutes then the temperature was increased at at rate of 8°C per minute to a final temperature of 275°C. The final temperature was held for 2 minutes. Nitrogen was used as the carrier gas.

In order to assess injection reproducibility and to allow comparisons between OTUs assayed at different times, internal standards were used. These included methyl laurate, methyl palmitate and methyl stearate. All OTU extracts were examined two times, once without and once with the internal standards.

In addition to the internal standards, a known mixture of bacterial FAME (Supelco) was analyzed in order to optimize peak separation and to make presumptive peak identifications. Identification was made on the basis of co-chromatographing peaks and identical retention times relative to methyl palmitate.

FAME Data Analysis

Peaks were classified by their retention times relative to methyl palmitate. Only those peaks present in 8 percent or more of the OTUs were included in the computer analysis. Peak areas were standardized by computing the percent area of a peak relative to the total peak area for each OTU extract. Peaks with areas below 0.1 percent of the total were excluded from the computation. Detection levels were far below this percentage for all OTUs. Peaks were coded into one to three binary digits depending on the range of relative percent areas for the peak over all OTUs. A "1" in the first digit represents 0.10 to 0.99 percent abundance within an OTU. A "1" in the second digit represents 1.00 to 9.99 percent abundance. A "1" in the third

digit represents 10 percent or greater abundance within an OTU. Peaks with one or two digits fell into first, and first and second percentage groups respectively. The total number of peaks and the total number of characters coded (binary digits) are shown in Table 11. A total of 55 peaks were coded into 113 characters. These characters are listed in Table 12. Presence was coded as "1" and absence as "0". Additive coding was used for the multistate characters (100).

Comparison of the Two Classification Systems

Inter- and Intragroup Similarities

Group characteristics from defined groups in both classification systems were calculated. Inter- and intragroup similarities also were calculated for each system. OTU occurrence in groups for each system was then compared between systems. Intergroup similarities were then related to OTU occurrences in both systems.

Computer Programs

The numerical taxonomic analysis was carried out using the TAXAN 5 and IGPS 3 program packages provided by Dr. R. R. Colwell, University of Maryland. The programs were rewritten for the DEC 10 computer at the University of Tennessee, Knoxville. Analyses using the SAS and SPSS program packages were performed using the IBM 370 and DEC 10 systems at the computer facility at the University of Tennessee, Knoxville.

Table 11. Number of peaks coded in the FAME analysis and the number of binary characters coded per peak

	Number of Character States	Number of Peaks	Digits per Peak	Number of Coded Characters
	1	10	1	10
	2	32	2	64
	3	13	3	39
Total Number of Peaks		55		
Total Number of Characters				113

Table 12. Percent positive frequency for FAME characters used in the computer analysis of the Saucon Creek OTUs

Character[a]	Percent	Character	Percent
-515B	4	20A	4
-515A	10	28B	4
-429B	4	28A	15
-429A	21	33C	2
-417	25	33B	25
-407	23	33A	35
-355	8	37B	6
-336C	2	37A	17
-336B	29	50B	2
-336A	52	50A	21
-295B	8	56C	14
-295A	46	56B	60
-289	10	56A	69
-280B	8	64C	6
-280A	17	64B	21
-250	19	64A	25
-195B	19	74B	35
-195A	33	74A	48
-185B	2	106B	15
-185A	19	106A	35
-165C	2	115B	2
-165B	60	115A	15
-165A	83	131C	54
-125B	12	131B	69
-125A	46	131A	71
-110	17	149C	4
-102C	25	149B	35
-102B	31	149A	79
-102A	37	168	15
-94B	6	179C	21
-94A	8	179B	31
-81B	35	179A	40
-81A	64	191B	2
-65B	14	191A	8
-65A	21	198B	21
-47B	15	198A	33
-47A	19	208C	4
-28B	15	208B	6
-28A	31	208A	18
-18C	56	215B	2
-18B	73	215A	21
-18A	75	242B	2
0C	56	242A	25
0B	98	265B	10
0A	100	265A	37
20B	4	276	15

Table 12. (Continued)

Character	Percent
285B	2
285A	29
306B	4
306A	17
323B	8
323A	42
336C	2
336B	8
336A	29
348B	8
348A	23
394	21
410B	14
410A	73
458B	8
458A	21
475	14
514B	6
514A	8
524B	2
524A	15

[a]Characters are retention times in seconds relative to methyl palmitate.

III. RESULTS

Site Variation and Data Reproducibility

Site Variation

The physical, chemical, and biological data were examined by discriminant analysis using site-time samplings as the unit of examination. The results of these analyses have been previously reported and are summarized here as they pertain to the present investigation (87). Temporal differences were found to have the greatest effect on the discriminant scores, although site differences also were discernible. This analysis was approximately 92 percent efficient in reclassifying the site-time samplings.

On the basis of the data set available (degrees freedom and the weight of the standardized variables on the discriminant functions) four variables were chosen for further study: total viable count, phenanthrene resistant bacteria count, ATP, and alkaline phosphatase. By using site-time discriminant analysis with these four variables, the reclassification efficiency was 59 percent. Site differences were observable within sampling times.

In order to examine the means of the individual variables, multiple analysis of variance followed by Duncan's multiple range testing was performed. Site, time, and site-time interaction were considered as main effects. Time was found to have a strong effect although site and site-time interaction also were found to have significant effects when tested at the $P = 0.95$ level. When the sites were examined within the June 1979 sampling date, however, no significant

differences could be observed for any of the variables using Duncan's multiple range test. As found in the discriminant analysis, time was found to have a major effect while the site effects were significant though much smaller. For the June 1979 sample, no individual variable was found to vary significantly between sites.

Reproducibility

The methods used in the morphological, physiological and biochemical portion of this study were identical to those used in a previous work (Part II). In that study using duplicate strains, a percent variation value, expressed as a similarity measure, was found to be 2 to 3 percent. This value would not significantly affect clustering (98, 99).

In the FAME portion of the study, error can develop at three levels: the extraction and methylation process, injection into the gas chromatograph, and coding the data. The injection error was found to be only 0.5 percent when expressed as an average of the error between paired peaks following expression of peak area as a percentage of the total area.

Peak presence or absence presented no data coding problem; however, peak area, a continuous, quantitative variable, presented problems. A three digit character coded in an additive fashion was chosen for a number of reasons. Intuitively classifying peaks as major (10 percent or greater of the total peak area), moderate (1.00 to 9.99 percent), or minor (0.10 to 0.99 percent) was suggested since in many previous studies bacterial fatty acids have been shown to demonstrate this major-minor peak area pattern (14, 32, 68). By choosing three

digit peak characters, weighting was partially avoided by allowing a maximum of three binary characters per peak. Even so, this minor weighting (3 percent of the total FAME characters per three digit peak) can easily be justified, in that some degree of quantification was necessary and that a major peak may be more meaningful than a minor peak when comarisons between OTUs are made (14, 32). Although some data were lost due to converting this continuous variable into discrete measures, accuracy was maintained.

When duplicates were compared using this system only a 1 to 3 percent variation was observed. This would not significantly affect clustering. It was assumed that the transformation of a continuous variable into a discrete variable masked a significant portion of the extraction and injection error while allowing for accuracy and reasonable precision.

Numerical Taxonomic Analyses

Numerical Taxonomic Examination of the Phenetic Data

A total of 49 OTUs (82 percent) was recovered in 12 groups defined at the 80 to 85 percent level using the S_{sm} coefficient. When examined using the S_J coefficient similarity values were lower, due to the absence of negative matching, but group membership remained the same. Some intragroup OTU shifting was noted. Higher order rearrangement, however, occurred with most of the defined groups. By using the S_J coefficient, four major separations were observed. These were (i) groups 1 and 2; (ii) groups 8, 9, 19, 12, 5 and 11; (iii) groups 7, 4 and 6; and (iv) group 3. These groupings were

related one to another at approximately the 33 percent similarity level. Higher order groupings for the S_{sm} coefficient using the phenetic data are shown on the simplified dendrogram (Fig. 7). Inter-OTU similarity values greater than 70 percent and defined groups are shown on the sorted similarity matrix (Fig. 8). Group 12, containing 10 OTUs, was the largest group recovered. Three other groups contained 6 to 7 OTUs each. The remaining 8 groups contained 2 to 3 OTUs each. From the dendrogram (Fig. 7) and the inter- and intragroup similarity matrix for the phenetic data (Fig. 9A), higher order relationships can be clearly defined for the S_{sm} coefficient. As shown in Figure 9A, groups 1, 2 and 3 have intergroup similarities between 70 and 80 percent. Groups 4 and 5, as well as 4 and 6, are shown to be between 70 and 80 percent similar. Although not shown, the intergroup similarity between groups 5 and 6 was found to be 64 percent. Groups 8, 9 and 10 were found to be 80 to 84 percent similar one to another. Groups 11 and 12 were 70 to 80 percent similar one to another, as well as to groups 8, 9 and 10. Although the values in Figure 9A do not, in all cases, match with the linkages shown in the dendrogram (Fig. 7), it should be noted that the Figures 7 and 9A are only two dimensional representations of multidimensional relationships, and that a significant amount of distortion is introduced when presented as such.

Group characteristics are given in Tables 13 and 14. Percent positive frequencies for the phenetic characters are shown in Table 10. As a whole the OTUs can be described as non-motile (83 percent), short to medium rods (2-7:1) (77 percent) which form raised (83 percent), smooth edged (79 percent), shiny (84 percent), off-white (70 percent),

Figure 7. A simplified dendrogram prepared from the phenetic data of the Saucon Creek OTUs. The S_{sm} coefficient was used. Clustering was achieved using the UPGMA technique.

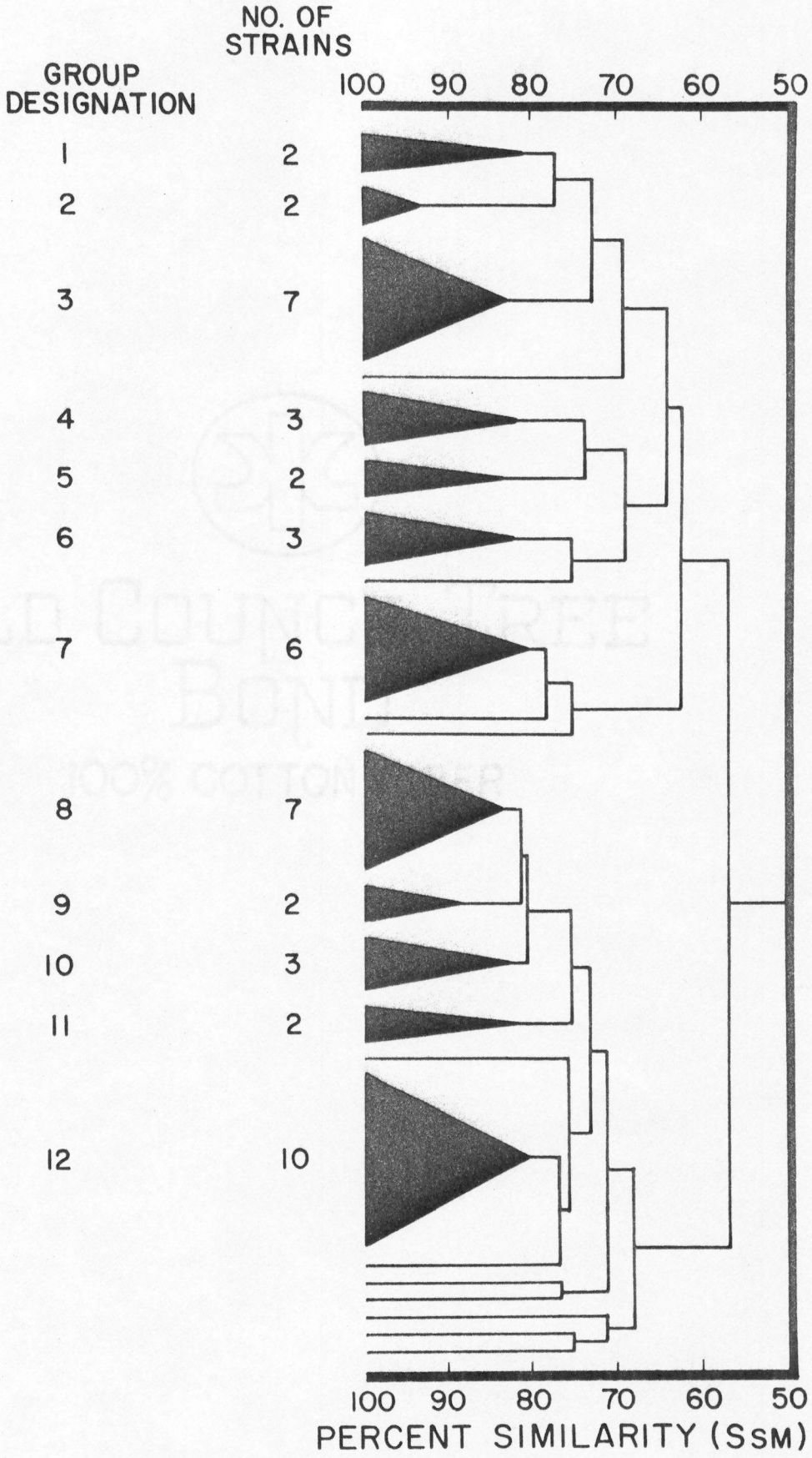

NO. OF STRAINS
GROUP DESIGNATION
100 90 80 70 60 50
1 2
2 2
3 7
4 3
5 2
6 3
7 6
8 7
9 2
10 3
11 2
12 10
100 90 80 70 60 50
PERCENT SIMILARITY (SSM)

Figure 7.

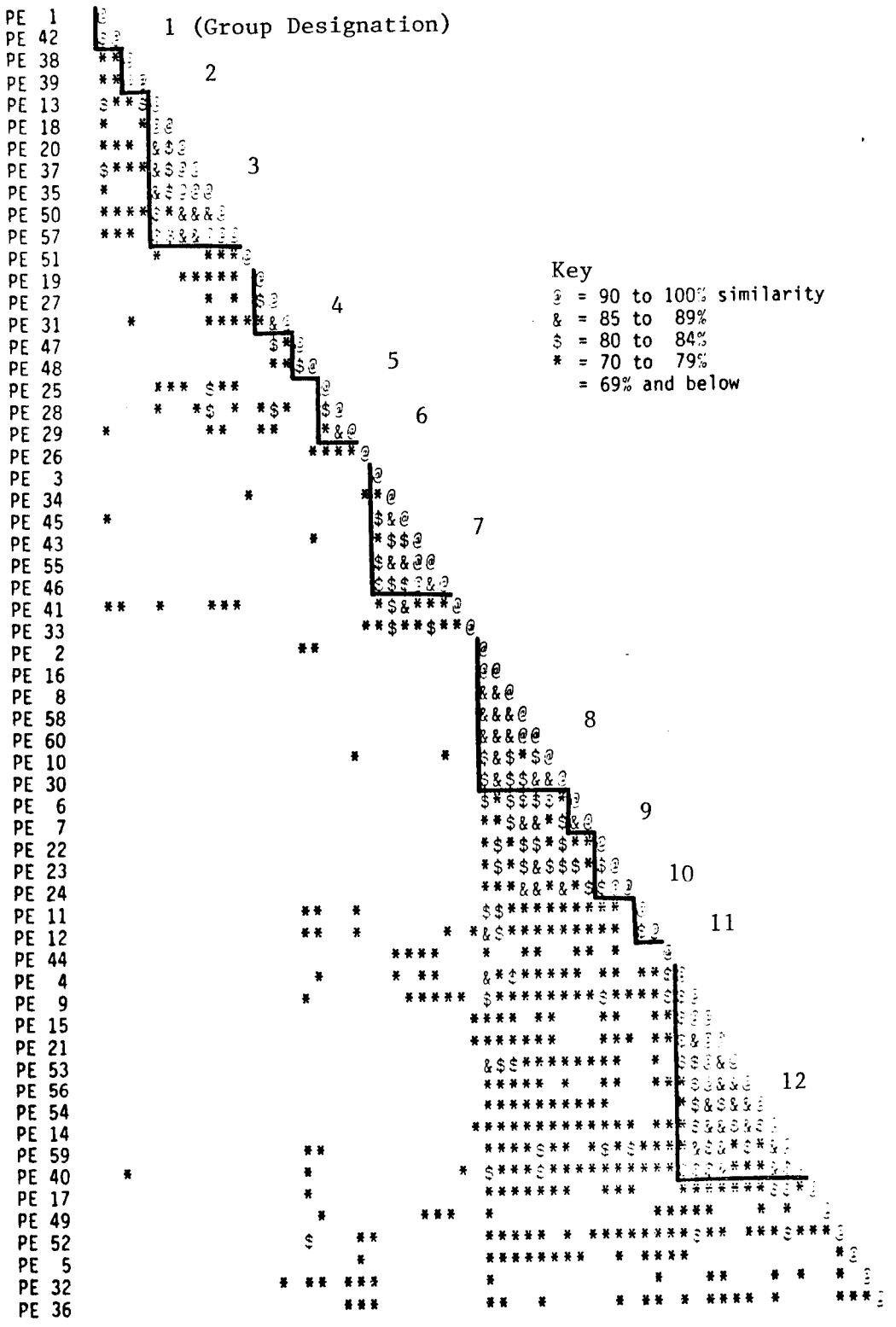
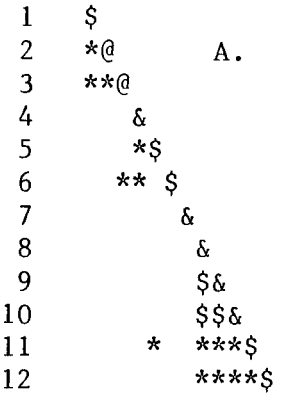


Figure 8. A sorted similarity matrix prepared from the phenetic data of the Saucon Creek OTUs.

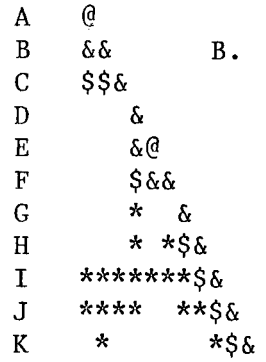


A. Phenetic Data

Key

@ = 90 to 100% similarity
& = 85 to 89%
$ = 80 to 84%
* = 70 to 79%
= less then 69%

Group Designations



B. FAME Data

Group Designations

Figure 9. Inter- and intragroup similarities for both the phenetic and FAME classification systems.

Table 13. Characteristics of groups 1 to 10 from the Saucon Creek OTUs

Character	Group Designation 1	2	3	4	5	6	7	8	9	10
Colonial Morphology										
elevation										
flat	-[a]	-	-	-	-	-	d[b]	-	-	-
peaked	-	-	-	d	-	-	d	-	-	-
raised	+[c]	+	+	d	+	+	d	+	+	+
edge										
crenate	-	-	-	-	-	-	+	-	+	-
smooth	+	+	+	+	+	+	-	+	-	+
surface										
shiny	+	+	+	+	+	+	+	d	-	d
waxy	-	-	-	-	-	-	-	d	+	d
transparency										
opaque	-	-	-	-	-	-	-	+	+	d
translucent	-	-	d	+	d	+	d	-	-	d
transparent	+	+	d	-	d	-	d	-	-	-
color										
off-white	d	-	d	+	+	-	+	+	+	+
yellow	d	+	d	-	-	+	-	-	-	-
pink	-	-	-	-	-	-	-	-	-	-
Micromorphology										
length to width ratio										
coccoid	-	-	d	d	d	-	-	-	-	-
1.5-2:1	+	+	d	+	+	-	-	-	d	-
2-4:1	+	d	d	d	-	+	+	d	d	d
5-7:1	d	-	d	-	-	+	+	+	+	+
7-12:1	-	-	-	-	-	-	d	-	-	d
filaments	-	-	-	-	-	-	d	-	-	d
cell characteristics										
branching	-	-	-	-	-	-	-	-	-	d
chaining	-	-	-	d	-	-	d	d	-	-
curved	-	+	d	-	-	d	-	d	+	d
granulation	-	-	-	-	-	d	-	d	d	d
pleomorphic	-	-	d	-	-	d	-	d	d	d
swelling	-	-	-	-	-	-	-	-	+	d
motility										
wet mount	-	-	-	-	-	-	d	-	-	-
Staining Reactions										
Gram stain[d]	+	var	var	var	-	var	-	+	+	var
Physiology										
salt tolerance										
2% NaCl	+	+	d	+	+	-	+	+	+	+
5% NaCl	+	+	-	-	d	-	-	+	+	+
resistance to inhibitors										
bismuth citrate	-	-	-	-	d	-	d	d	-	d
Aroclor 1254	d	-	d	+	+	+	d	+	+	+

Table 13. (Continued)

Character	Group Designation 1	2	3	4	5	6	7	8	9	10
growth at temperature										
4°C	–	–	–	–	–	–	–	d	d	+
15°C	+	+	d	+	+	+	+	+	+	+
37°C	–	+	–	d	d	d	d	–	–	–
42°C	–	–	–	d	–	–	d	–	–	–
survival at temperature										
42°C	–	+	–	d	–	d	d	d	d	+
Biochemistry										
catalase	+	+	d	+	+	+	+	+	+	+
nitrate reduction	d	–	+	–	d	+	+	d	+	–
nitrite reduction	–	–	–	–	d	–	–	d	d	–
oxidase	–	–	–	–	–	d	d	d	–	–
glucose metabolism										
alkaline reaction	–	–	+	+	–	d	–	–	–	–
fermentative	+	+	d	–	–	–	+	d	d	d
oxidative	–	–	d	+	–	d	–	d	d	d
no reaction	–	–	–	–	+	d	–	–	–	–
degradation of										
casein	+	–	–	–	–	–	+	–	–	–
DNA	+	+	d	–	–	–	d	–	–	–
hypoxanthine	–	–	–	–	–	–	–	–	–	–
starch	–	–	d	–	–	–	+	–	–	–
Tween 80	–	+	d	+	+	–	+	+	+	+
tyrosine	+	+	–	d	d	–	+	d	+	+
use as sole carbon source										
adonitol	–	–	–	–	+	–	–	d	–	–
alanine	–	–	–	+	+	–	d	+	+	+
arabinose	–	–	–	–	+	–	d	d	+	–
arginine	–	–	–	d	+	–	d	+	+	+
butanol	–	–	–	+	+	d	–	+	+	+
cellobiose	d	–	–	d	d	d	d	d	+	+
galactose	d	+	–	d	+	d	+	+	+	d
glucose	+	+	–	–	+	d	+	+	+	+
glycerol	+	+	d	d	+	d	+	+	+	+
glycogen	–	–	–	–	+	–	–	d	–	–
inositol	–	–	–	–	–	–	–	+	+	+
lactose	d	–	–	d	–	–	–	d	+	+
leucine	–	–	–	d	d	d	d	+	+	+
lysine	–	–	–	–	–	–	–	d	+	+
mannitol	d	d	–	d	–	–	d	+	+	+
melezitose	d	–	–	d	+	–	d	d	–	+
melibiose	–	–	–	d	+	–	–	d	+	+
propanol	–	d	–	+	+	d	–	+	+	+
ribose	+	–	–	–	+	d	+	+	+	+
salicin	–	+	d	–	–	d	d	+	+	d
sodium acetate	–	–	–	+	+	+	+	+	+	+

Table 13. (Continued)

Character	Group Designation									
	1	2	3	4	5	6	7	8	9	10
sodium aspartate	+	-	-	+	+	+	d	+	+	+
sodium butyrate	-	-	-	-	-	-	-	d	+	+
sodium citrate	-	+	-	+	d	d	d	+	+	+
sodium glutamate	-	-	-	+	+	+	+	+	+	+
sodium lactate	d	-	-	d	+	+	d	+	+	+
sodium pyruvate	+	-	-	+	+	+	d	+	+	+
sodium succinate	d	-	-	+	+	+	+	+	+	+
serine	-	-	-	-	-	-	+	+	+	+
sucrose	d	-	d	-	+	d	+	+	+	+
tryptophan	d	-	-	-	d	-	d	d	-	+
xylose	d	+	-	-	d	-	d	+	+	+

[a]- = less than 10 percent positive.

[b]d = 10 to 89 percent positive.

[c]+ = 90 to 100 percent positive.

[d]For the Gram stain only, - = Gram negative, var = Gram variable and + = Gram positive.

Table 14. Characteristics of groups 11 and 12 from the Saucon Creek OTUs

Character	Group Designation 11	12
Colonial Morphology		
elevation		
flat	-[a]	-
peaked	-	-
raised	+[b]	+
edge		
crenate	-	-
smooth	+	+
surface		
shiny	+	+
waxy	-	-
transarency		
opaque	+	d[c]
translucent	-	d
transparent	-	d
color		
off-white	d	d
yellow	d	d
pink	-	-
Micromorphology		
length to width ratio		
coccoid	-	-
1.5-2:1	-	d
2-4:1	+	+
5-7:1	+	+
7-12:1	-	d
filaments	-	-
cell characteristics		
branching	-	-
chaining	-	-
curved	-	d
granulation	d	-
pleomorphic	-	d
swelling	-	d
motility		
wet mount	-	-
Staining Reactions		
Gram stain[d]	var	-
Physiology		
salt tolerance		
2% NaCl	+	+
5% NaCl	d	+
resistance to inhibitors		
bismuth citrate	-	-
Aroclor 1254	+	+

Table 14. (Continued)

Character	Group Designation 11	12
growth at temperature		
4°C	-	d
15°C	+	+
37°C	-	d
42°C	-	-
survival at temperature		
42°C	-	d
Biochemistry		
catalase	+	+
nitrate reduction	-	+
nitrite reduction	-	d
oxidase	d	-
glucose metabolism		
alkaline reaction	-	-
fermintative	d	d
oxidative	-	-
no reaction	d	d
degradation of		
casein	-	+
DNA	-	d
hypoxanthine	-	d
starch	-	d
Tween 80	+	-
tyrosine	-	+
use as sole carbon source		
adonitol	d	d
alanine	+	+
arabinose	+	+
arginine	d	+
butanol	+	+
cellobiose	+	+
galactose	+	+
glucose	+	+
glycerol	+	+
glycogen	d	d
inositol	d	+
lactose	-	+
leucine	+	d
lysine	+	+
mannitol	+	+
melezitose	d	+
melibiose	d	+
propanol	+	+
ribose	+	+
salicin	d	+
sodium acetate	+	+

Table 14. (Continued)

Character	Group Designation 11	12
sodium aspartate	d	+
sodium butyrate	-	-
sodium citrate	+	+
sodium glutamate	+	+
sodium lactate	+	+
sodium pyruvate	+	+
sodium succinate	+	+
serine	+	+
sucrose	+	+
tryptophan	-	d
xylose	+	+

[a]- = less than 10 percent positive.

[b]+ = 90 to 100 percent positive.

[c]d = 10 to 89 percent positive.

[d]For the Gram stain only, - = Gram negative and var = Gram variable.

colonies on the basal test medium. Most of the OTUs (55 percent) tolerated 5 percent sodium chloride and could be described as low temperature mesophiles with 95 percent of the strains growing at 15°C. Although only 8 percent of the strains grew at 42°C, over 42 percent were able to survive 42°C for 5 days and exhibit growth at 20°C following exposure. As was expected from the previous study (Part II), 85 percent of the OTUs grew in the presence of high concentrations of PCB. Almost all of the OTUs were catalase positive (94 percent) and 60 percent reduced nitrate to nitrite, however, only 8 percent could reduce nitrite under the test conditions. Glucose metabolism was predominately fermentative (62 percent). Only 2 of the 6 compounds tested, Tween 80 and tyrosine, were degraded by over 50 percent of the OTUs. In comparison, 24 of the 32 sole carbon sources were utilized by 50 percent or more of the OTUs. A total of 12 carbon sources were utilized by 70 percent or more of the OTUs. Of these 12, 5 were organic acids, 4 were mono- or disaccharides, 2 were amino acids and 1 was a sugar alcohol. All but 1 of the carbon sources were used by 20 percent or more of the OTUs.

From the group characteristics presented in Tables 13 and 14, certain trends relating to higher order relationships can be noted. In groups 1 through 7 colonial transparency was either translucent or transparent, while in groups 8 through 12, with only 1 exception, colonies were opaque or translucent. Shorter rod to cocci dominated in groups 6 through 12. The most significant differences relating to higher order relationships were observed in the sole carbon source characters. Clear divisions can be seen between groups 1 through 3,

groups 4 through 7, and groups 8 through 12. Groups 1 through 3 had the potential to utilize 8.6 ± 6 carbon sources per group (potential carbon sources being the sum of "+" and "d" within a group), groups 4 through 7 potentially could use 21 ± 3.7, and groups 8 through 12 were capable of utilizing 30 ± 1 carbon sources. These divisions relate closely to those shown in Figure 7. It is clear that while representing only a little over one third of the coded characters, carbon source characters exerted a strong influence on both group formation and higher order relationships when examined by the S_{sm} coefficient.

Numerical Taxonomic Examination of the FAME Data

A total of 11 groups containing 45 OTUs (87 percent) were recovered at the 85 to 90 percent level using the S_{sm} coefficient. When examined using the S_J coefficient, group memberships remained the same with one exception, OTU PE 52, located next to group J (Fig. 10), was not recovered in the group. Although similarity values were lower, higher order (intergroup) relationships remained essentially unchanged. A small dgree of intragroup OTU rearrangement was observed. Small groups, containing 2 to 3 OTUs per group, made up 6 of the groups. The remaining 5 groups contained 5 to 7 OTUs each.

Strong higher order groupings were observed. In both the simplified dendrogram (Fig. 11) and the sorted similarity matrix (Fig. 10) clear separations can be observed between the sets of groups A, B, and C; D, E, and F; and G, H, I, J, and K. Although less clear, it can be observed from the FAME data inter- and intragroup similarity matrix (Fig. 9B), that while groups G, H, I, J, and K form a chain-like series, groups G and H, and group K are only slightly (below 70 percent) similar.

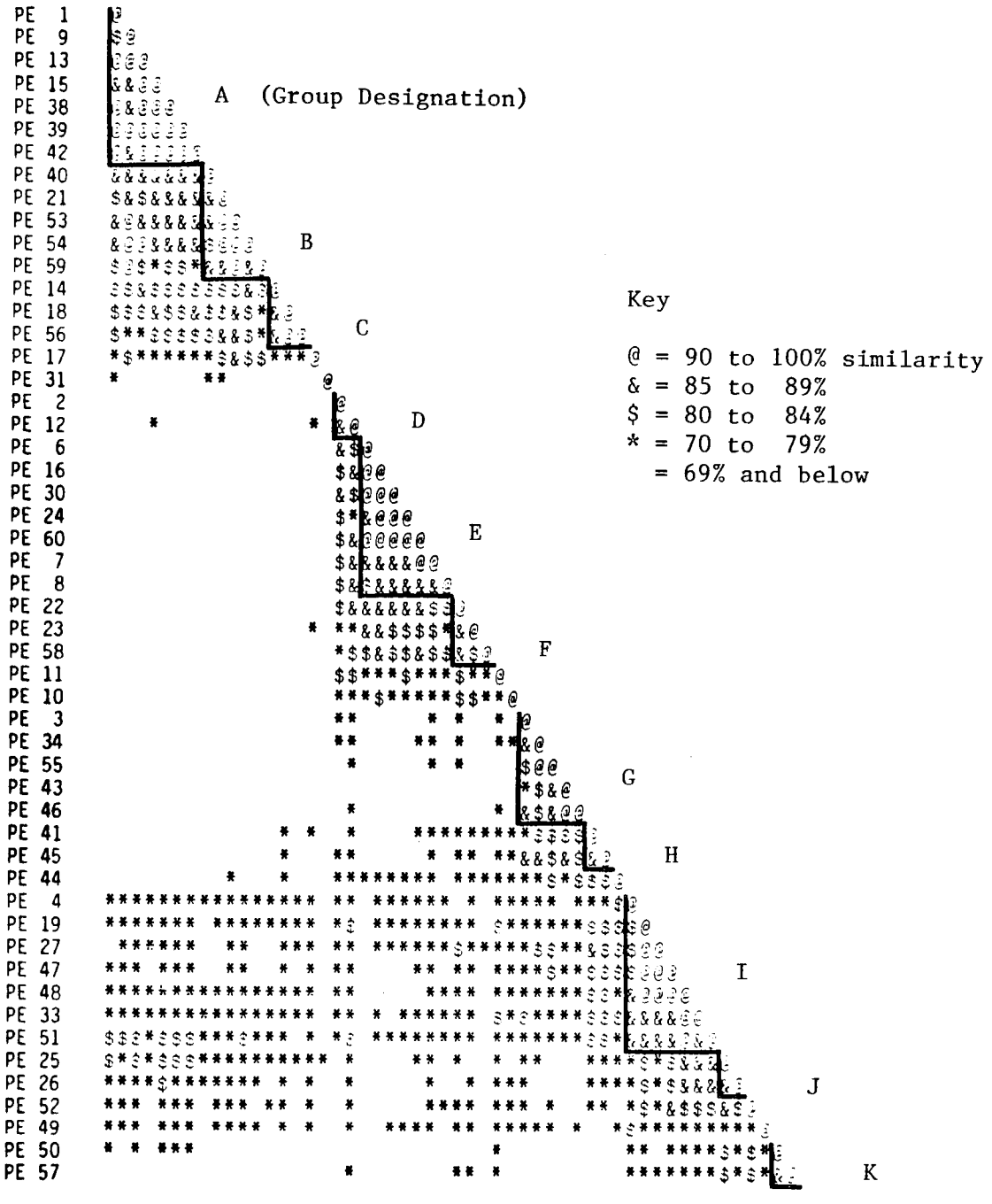


Figure 10. A sorted similarity matrix prepared from the FAME data of the Saucon Creek OTUs.

Figure 11. A simplified dendrogram prepared from the FAME data of the Saucon Creek OTUs. The S_{sm} coefficient was used. Clustering was achieved using the UPGMA technique.

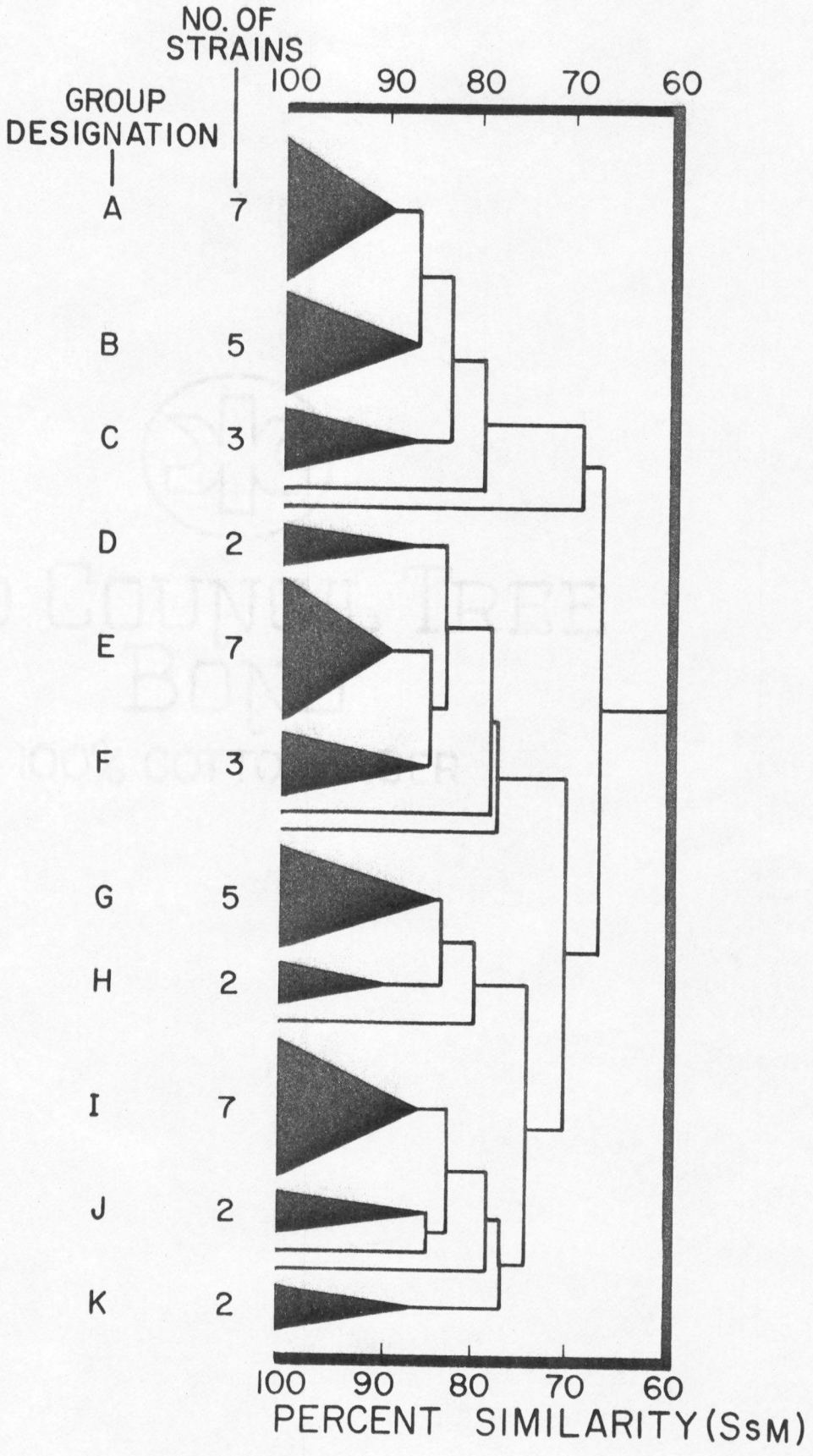

NO. OF STRAINS
GROUP DESIGNATION
100 90 80 70 60
A 7
B 5
C 3
D 2
E 7
F 3
G 5
H 2
I 7
J 2
K 2
100 90 80 70 60
PERCENT SIMILARITY (SsM)

Figure 11.

Group characteristics are given in Table 15. The percent positive frequencies for the FAME characters are given in Table 12. Of the 55 peaks examined in this study, only 9 (-336, -165, -81, -18, 0, 56, 131, 149, and 410 sec) were detected in 50 percent or more of the OTUs. Only the 0 sec character (methyl palmitate) was present in all OTUs. Tentative identifications of these peaks were made. The respective identificaiton of each peak was methyl laurate, methyl myristate, methyl pentadecanoate, methyl palmitoleate, methyl palmitate, methyl heptadecanoate, methyl stearate and an unknown. Presumptive identification of the remaining peaks was not attempted. Although necessary for identification of the clustered OTUs, the chemical identity of the FAME peaks is not necessary for classification. Chromatograms for OTUs PE 1 and PE 55, representing a simple and complicated FAME pattern respectively, are shown in Figures 12 and 13 respectively.

Comparisons between the Two Classifications

OTU Recovery

The occurrence of OTUs within groups in both classification systems is shown in Table 16. Members of group 3, group 6, and 3 unclustered OTUs did not grow or grew poorly on the growth medium for the FAME analysis. There was no complete correspondence between and two groups between classification systems. Only 4 OTUs were unclustered in both systems. Recovery of an OTU in a group in one system did not guarantee recovery in the other system. Four OTUs (PE 41, PE 33, PE 51 and PE 26) recovered in groups H, I and J in the FAME system were not

Table 15. Characteristics of groups A to K from the Saucon Creek OTUs

Character[a]	Group Designation										
	A	B	C	D	E	F	G	H	I	J	K
-515B	–[b]	–	–	–	–	–	d[c]	–	–	–	–
-515A	–	–	–	–	–	–	d	–	–	–	–
-429B	–	–	–	–	–	–	–	–	–	–	–
-429A	–	–	–	–	d	d	d	d	d	d	–
-417	–	–	–	–	–	+[d]	d	d	d	d	+
-407	–	–	–	–	d	d	d	–	–	–	d
-355	–	–	–	–	–	–	d	–	–	–	–
-337C	–	–	–	–	–	–	–	–	d	–	–
-337B	–	–	d	–	–	–	+	+	d	–	–
-337A	–	–	d	–	d	d	–	–	–	–	d
-295B	–	–	–	–	–	–	–	–	d	–	–
-295A	–	–	–	d	d	d	+	+	d	+	d
-289	–	–	–	–	d	–	–	–	–	–	d
-280B	–	–	–	–	–	–	d	d	–	–	–
-280A	–	–	d	–	–	–	d	d	–	–	–
-250	–	–	–	d	–	d	d	+	–	–	–
-195B	d	d	+	–	–	–	–	–	–	–	–
-195A	d	d	–	–	–	d	d	–	–	–	–
-185B	–	–	–	–	–	–	–	–	–	–	–
-185A	–	–	–	–	d	d	+	+	–	–	–
-165C	–	–	–	–	–	–	–	–	–	–	–
-165B	d	d	d	+	+	+	+	+	d	–	–
-165A	d	d	d	–	–	–	–	–	d	d	d
-125B	–	–	–	–	–	–	–	–	d	–	–
-125A	d	d	d	–	–	–	+	+	d	–	–
-110	–	–	–	–	–	–	+	+	d	–	–
-102C	d	+	–	–	–	–	–	–	–	–	–
-102B	d	–	–	–	–	–	–	–	d	–	–
-102A	–	–	–	–	–	d	–	–	–	–	d
-94B	–	–	+	–	–	–	–	–	–	–	–
-94A	–	d	–	–	–	–	–	–	–	–	–
-81B	d	–	–	d	d	+	d	+	–	–	–
-81A	d	d	d	d	d	–	d	–	d	–	–
-65B	d	d	d	–	–	–	–	–	–	–	–
-65A	–	d	–	–	–	–	–	–	d	–	–
-47B	–	d	d	–	–	–	–	–	–	–	–
-47A	d	d	–	–	–	–	–	–	–	–	–
-28B	d	d	d	–	–	–	–	–	–	–	–
-28A	d	d	d	–	–	–	–	–	–	–	–
-18C	–	–	–	+	d	+	+	+	+	–	–
-18B	d	d	–	–	d	–	–	–	–	d	–
-18A	–	–	–	–	–	–	–	–	–	–	d
0C	–	d	–	+	+	+	+	+	d	–	–
0B	+	d	d	–	–	–	–	–	d	+	+
0A	–	–	d	–	–	–	–	–	–	–	–

Table 15. (Continued)

Character	Group Designation										
	A	B	C	D	E	F	G	H	I	J	K
20B	–	–	–	–	–	–	d	–	–	–	–
20A	–	–	–	–	–	–	–	–	–	–	–
28B	–	–	–	–	–	–	d	–	–	–	–
28A	–	–	–	–	–	–	d	+	–	–	–
33C	–	–	–	–	–	–	–	–	–	–	–
33B	–	d	d	d	+	d	–	–	–	–	–
33A	d	–	–	d	–	d	–	–	–	–	–
37B	d	d	–	–	–	–	–	–	–	–	–
37A	d	d	d	–	d	–	–	–	–	–	–
50B	–	–	–	–	–	–	d	–	–	–	–
50A	d	–	–	–	–	–	d	d	d	d	–
56C	d	d	–	–	–	–	–	–	–	–	–
56B	–	d	+	+	+	+	–	d	d	–	–
56A	–	–	–	–	–	–	–	–	d	–	–
64C	–	–	–	–	–	–	–	–	d	–	–
64B	–	d	–	–	d	d	–	–	–	–	–
64A	–	–	–	–	–	–	–	–	–	–	–
74B	–	–	–	d	d	+	d	+	d	–	–
74A	–	–	–	d	d	–	–	–	d	–	d
106B	–	–	–	d	d	d	–	–	–	–	–
106A	–	–	d	d	d	d	d	–	–	–	–
115B	–	d	–	–	–	–	–	–	–	–	–
115A	d	d	d	–	–	–	d	–	d	–	–
131C	–	–	–	+	d	+	d	–	+	+	+
131B	–	–	–	–	d	–	d	+	–	–	–
131A	–	–	–	–	–	–	–	–	–	–	–
149C	–	–	–	–	–	–	–	–	–	–	+
149B	d	–	–	d	d	d	d	+	d	+	–
149A	d	d	d	d	d	d	d	–	–	–	–
168	–	–	–	–	–	–	+	–	–	–	+
179C	–	–	–	+	+	d	–	–	–	–	–
179B	–	–	–	–	–	d	d	–	–	–	–
179A	–	–	–	–	–	–	d	–	–	d	–
191B	–	–	–	–	–	–	d	–	–	–	–
191A	–	–	–	–	–	–	d	–	–	–	+
198B	–	–	–	+	d	+	–	–	–	–	–
198A	d	–	–	–	d	–	–	+	–	–	–
208C	–	–	–	–	–	–	–	–	–	–	+
208B	–	–	–	–	–	–	–	–	–	–	–
208A	d	d	d	–	–	–	–	–	–	+	–
215B	–	–	–	–	–	–	–	–	–	–	–
215A	–	–	d	–	d	+	d	–	–	–	–
242B	–	–	–	d	–	–	–	–	–	–	–
242A	–	–	–	d	+	d	d	–	–	–	–
265B	–	–	–	d	–	–	–	–	–	–	–
265A	–	d	–	d	+	+	d	–	–	–	–

Table 15. (Continued)

Character	Group Designation										
	A	B	C	D	E	F	G	H	I	J	K
276	–	–	–	–	–	–	d	d	d	d	–
285B	–	–	–	d	–	–	–	–	–	–	–
285A	–	–	–	d	d	+	d	–	d	–	d
306B	–	–	–	–	–	–	–	–	–	–	d
306A	–	–	–	–	d	d	d	–	–	–	d
323B	–	–	–	–	–	–	–	d	–	–	d
323A	–	–	–	d	–	+	d	d	d	+	d
336C	–	–	–	d	–	–	–	–	–	–	–
336B	–	–	–	–	d	–	d	d	–	–	–
336A	–	–	–	–	d	+	d	–	–	–	–
348B	–	–	–	–	d	d	–	–	–	–	–
348A	–	–	–	–	d	d	–	–	–	–	d
394	–	d	–	–	d	+	–	–	–	–	d
410B	–	–	–	–	d	–	–	d	d	d	d
410A	d	d	–	+	d	+	+	–	d	d	d
458B	–	–	–	–	d	d	–	–	–	–	–
458A	–	–	–	–	d	d	–	–	–	d	–
475	–	–	d	–	d	d	–	–	–	–	–
514B	–	–	–	–	d	–	–	–	–	d	–
514A	–	–	–	–	–	–	–	–	–	–	–
524B	–	d	–	–	–	–	–	–	–	–	–
524A	–	–	–	+	d	d	–	–	–	–	d

[a]Characters are retention times in seconds relative to methyl palmitate. A blank or an "A" indicates a relative abundance of 0.1 to 1.0 percent, a "B" indicates a relative abundance of 1.0 to 9.9 percent and a "C" indicates a relative abundance of 10 percent or greater.

[b]– = less then 10 percent positive

[c]d = 10 to 89 percent positive

[d]+ = 90 to 100 percent positive.

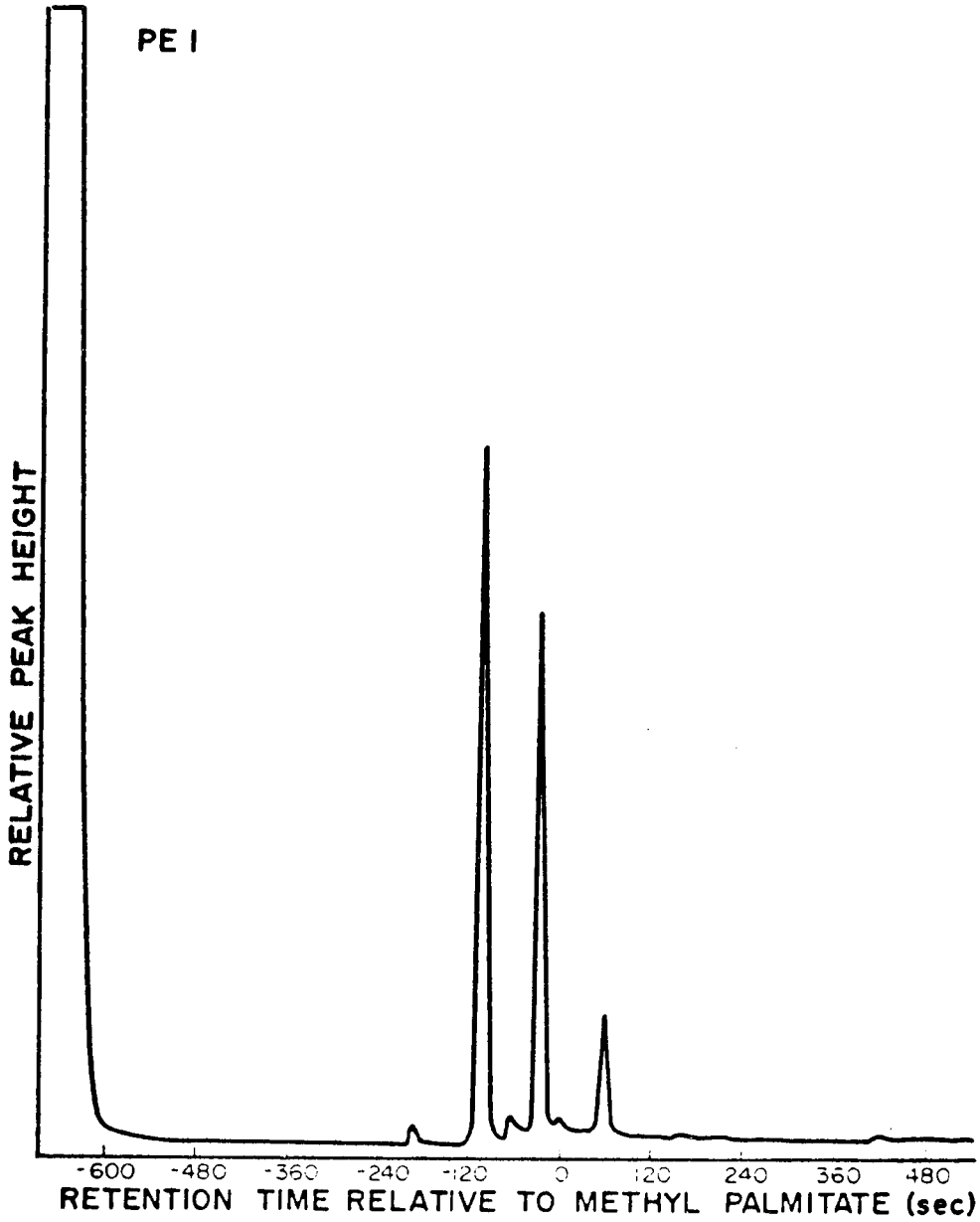


Figure 12. A simple gas chromatogram of the FAME extract of OTU PE 1.

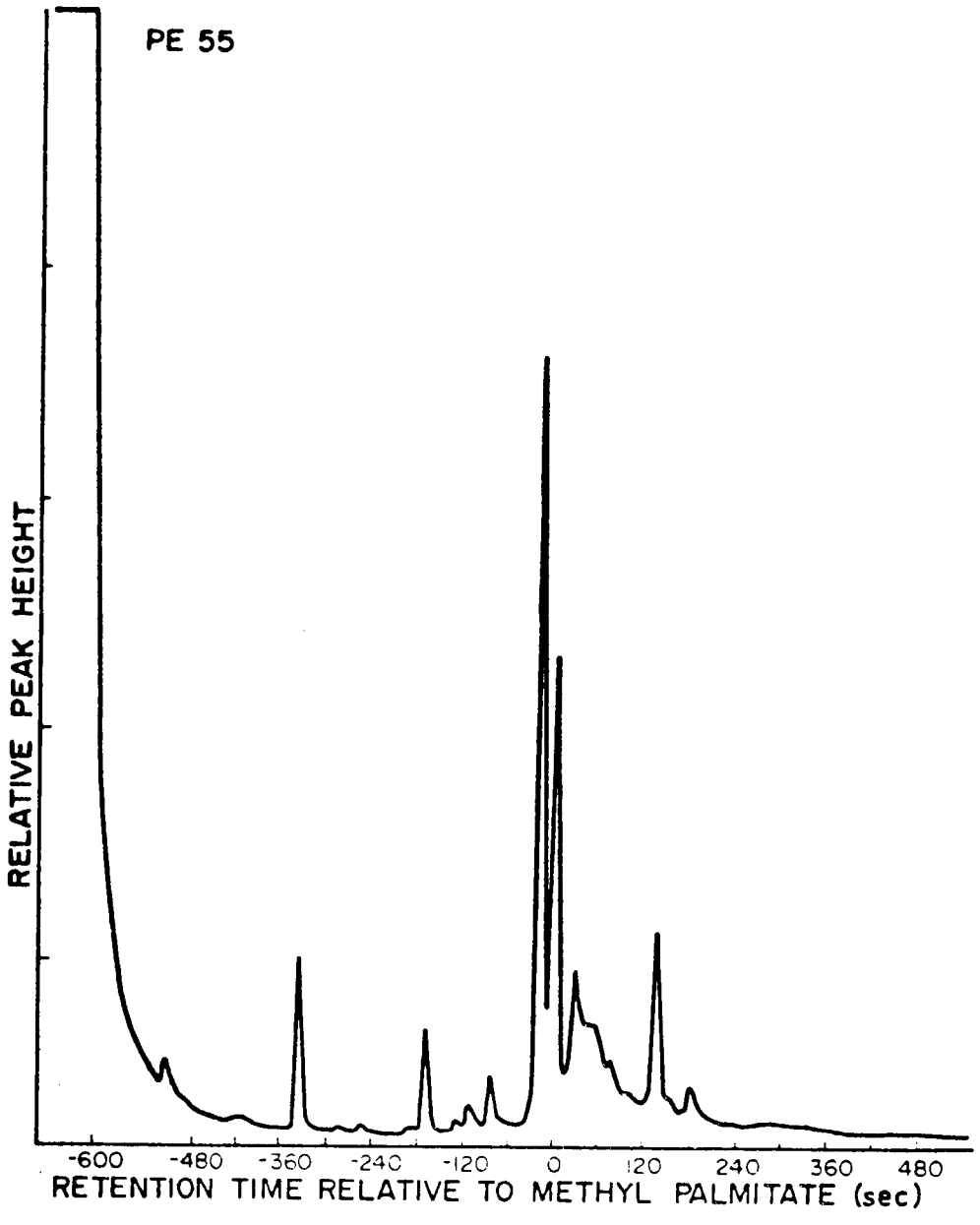


Figure 13. A complex gas chromatogram of the FAME extract of OTU PE 55.

Table 16. Occurrence of OTUs in both classification systems

Group Designation	Group Designation													
	A	B	C	D	E	F	G	H	I	J	K	XC[a]	NC[b]	Totals
1	2	-[c]	-	-	-	-	-	-	-	-	-	-	-	2
2	2	-	-	-	-	-	-	-	-	-	-	-	-	2
3	1	-	1	-	-	-	-	-	-	-	2	-	3	7
4	-	-	-	-	-	-	-	-	2	-	-	1	1	3
5	-	-	-	-	-	-	-	-	2	-	-	-	-	2
6	-	-	-	-	-	-	-	-	-	1	-	-	2	3
7	-	-	-	-	-	-	5	1	-	-	-	-	-	6
8	-	-	-	1	4	1	-	-	-	-	-	1	-	7
9	-	-	-	-	2	-	-	-	-	-	-	-	-	2
10	-	-	-	-	1	2	-	-	-	-	-	-	-	3
11	-	-	-	1	-	-	-	-	-	-	-	1	-	2
12	2	5	2	-	-	-	-	-	1	-	-	-	-	10
XC	-	-	-	-	-	-	-	1	2	1	-	4	3	
Totals	7	5	3	2	7	3	5	2	7	2	2	3	8	

[a] XC = OTUs not recovered in groups.

[b] NC = OTUs not included in the FAME classification.

[c] - = no OTUs present.

recovered in groups in the phenetic system. Likewise, 3 OTUs (PE 31, PE 10 and PE 11) recovered in groups 4, 8 and 11 respectively in the phenetic system were not recovered in the FAME defined groups.

Group Comparisons

There was no discernable trend with respect to groups splitting or lumping from one system to the other. Four phenetic groups were recovered entirely within FAME groups: groups 1 and 2 recovered in group A, group 5 recovered in group I, and group 9 recovered in group E. All these phenetic groups were small, i.e., containing only 2 OTUs per group. Three FAME groups were recovered solely within phenetic groups. Groups B and G, which both contain 5 OTUs, were recovered in phenetic groups 12 and 7 respectively. A smaller FAME group containing only 2 members (group K) was recovered in group 3. In most cases, groups from both systems split with OTUs being recovered in two or more groups in the other system, e.g., group 12 split into groups A, B, C and I, and group A split into groups 1, 2, 3 and 12. In many cases, however, a group complex was definable. These complexes split approximately along divisions defined by the higher order groupings. These were (i) groups 1, 2, 3 and 12 with groups A, B and C; (ii) groups 8, 9 and 10 with groups D, E and F; (iii) group 7 with groups G and H; and (iv) groups 4 and 5 with group I. Groups J and K, which contain 2 members each, seemed to correspond to groups 6 and 3 respectively, however, the loss of 3 OTUs from group 3 and the loss of 2 OTUs from group 6 from the FAME analysis precludes confirming any group correspondence.

IV. DISCUSSION

Discriminant analysis and multivariate analysis of variance were able to demonstrate strong temporal differences in the microbial community in the sediment of Saucon Creek. Significant site differences were found to exist within the temporal variation. Despite the significant site variation, which was demonstrated using multivariate analysis of variance, no single factor was found to vary significantly between the sample sites for the June 1979 sampling.

In many cases temporal variations in microbial populations are thought to be directly related to temperature (60, 63, 81, 93). Spatial variation within an aquatic community, in the horizontal plane, has been little studied (57). Jones (57) was able to demonstrate significant differences between profundal and littoral lake sediments. Variation within the two zones was noted when sampling different lakes, however, zone variation within a zone for a single lake was not discussed.

Because site variation occurred within a much larger temporal variation, and because physical and chemical parameters were not found to differ significantly between sites within the June 1979 sampling, OTUs collected from the sites were treated as a sampling of the bacterial guild from a single aquatic community. A more detailed analysis of the microbial community of Saucon Creek sediments was carried out by Sayler (87).

The results of the phenetic numerical taxonomic classification were typical of those studies based on OTUs recovered directly from

the natural environment (71). The groups were well defined and a wide range of intergroup similarities indicated both highly related and unrelated phena. Higher order relationships among the groups which were apparent when examined with the S_{sm} coefficient, did not hold up when examined with the S_J coefficient. Certain intergroup relationships, however, were maintained, e.g., groups 1 and 2, and groups 8, 9 and 10. This sort of group shuffling is common (3, 71). Sole carbon source characters were of prime importance in group formation and, to some extent, in higher order relationships. Characters considered of basic importance to identification, particularly Gram stain and glucose metabolism, were often variable within groups. This was noted in an earlier study (Part II) and has been a normal result in many studies (3, 62, 71).

The physiological growth ranges of a heterotrophic bacterial species (e.g., salt tolerance, temperature range, pH range, potential carbon and energy sources, nutrient requirements, presence of resting forms, and other growth parameters) define the potential roles of the species. These potential roles have been termed the fundamental niche (52). The phenetic characterization of a bacterial population is a direct estimate of the populations fundamental niche. The fundamental niche is by definition quite large. Many taxa have overlapping fundamental niches. The actual role of a population in an environment, its realized niche, consists of only a small part of its fundamental niche (52). Under conditions of intense competition, such as those found in chemostat cultures (36, 66, 106), there is one species or perhaps sub-species per niche.

The definition of the term species, as used in this study, is the definition proposed by Gordon (42). That is, a species consists of a population of similar OTUs collected at different times from as many different environments as possible. Species, by this definition, is concentration dependent.

In a previous study (Part II) it was suggested that taxonomically different populations could occupy the same niche, if (i) their fundamental niches overlap, (ii) they both were physically present, and (iii) the nature of the environment was such that competition was limited. Carbon source utilization patterns were suggested to be of prime importance in niche structure within the bacterial guild. Allison et al. (2) described populations from pig cecum and colon soley on the basis of sole energy (carbon) sources. Baleux (7) found that biodegradation of surface active agents was accompanied by a large decease in bacterial guild diversity. Selection through manipulation of carbon and energy sources in growth media is a common microbiological tool.

Fatty acid methyl ester analysis of bacteria is a well established taxonomic technique for classifying bacteria (68, 89). Although not as conservative as ribosomal ribonucleic acid or certain proteins, such as cytochrome c, fatty acids, due to their critical roles in an organism, are somewhat conservative. Kaneda (59) proposed three types of lipid synthesizing systems within bacteria based on the enzyme systems involved and the characteristics of these systems. Although possible exceptions to the above occur, evolutionary relationships, and thereby genetic relationships, may be indirectly measured by using FAME analysis.

The methods for extraction and analysis of bacterial fatty acids have been well established. However, the subsequent methods used to estimate taxonomic relationships are still in an early stage of development. Many comparisons have been made intuitively and by eye comparisons of chromatograms (100). Recently, mathematical estimates of relatedness and cluster analysis have been used to estimate taxonomic relationships from FAME data (14, 32). In both of these studies correlation coefficients were used to estimate inter-OTU relationships. Boe and Gjerde (14) recommended normalizing raw data to the peak area of methyl palmitate, using a logarithmic transformation of the normalzed data, and estimating inter-OTU relationships by using the R correlation coefficient of Sokal and Michener (101). Clustering was achieved by using unweighted average linkage. This technique differentiated between Enterobacteriaceae and Vibrionaceae below the 0.8 level. Generic level separations within the Enterobacteriaceae were made at high levels, 0.94 to 0.97. It should be noted that character weighting occurs with this method and is directly related to the peak area of methyl palmitate. An OTU with a small methyl palmitate peak would have a greater chance of having a large number of large peaks than an OTU with a large methyl palmitate peak. Drucker (34), by using peaks normalized to total peak area and the Bravais-Pearson coefficient of linear correlation, was able to differentiate between Lancefield groups within the genus Streptococcus. The Bravais-Pearson coefficient places greater weight on major than on minor peaks.

In this study the data were normalized to total peak area. Similarity was estimated using association coefficients rather than

correlation coefficients. This necessitated the coding of the normalized data into a binary code. Character weighting was unavoidable due to the continuous nature of the peak area characters. Peaks were coded into a maximum of three digits thereby limiting weighting, and extraction and analysis error. However, additive coding was used based on the results presented by Drucker (34) and Boe and Gjerde (14) which demonstrated the importance of major peaks in classification.

The numerical taxonomic analysis yielded a classification which was sound. This soundness was based on minimal test error, a wide range of calculated similarities for both the S_{sm} and S_J coefficients, and the conservation of intragroup structure when analyzed using the two coefficients. Through the use of replicate extractions and three internal standards, data were well controlled.

FAME groups were defined at a relatively high level (85 to 90 percent using the S_{sm} coefficient). Group definition could easily be made at lower levels, however, the results of the S_J coefficient analysis suggest that the high level group definition is valid and not due to effects of negative matching. The group characteristics given in Table 15 are presented using non-additive coding so that peak area variation within a defined group can be assessed.

Both classifications yielded similar results in that 11 and 12 groups, containing 87 and 82 percent of the OTUs, were recovered in the FAME and phenetic classifications respectively. Although this correspondence can be attributed to the selection of similarity levels for group definition, these levels were determined independently for each classification system. Results from other numerical taxonomic studies

for both FAME and phenetic data also were considered. The 80 to 85 percent similarity (S_{sm}), selected for the phenetic classification, is a common level of group definition in both strictly taxonomic studies (44, 108) and ecological taxonomic studies (1, 3, 71). Although not directly comparable, the high level of correlation used to define taxa in studies using FAME patterns (14, 34) was considered when defining groups at the 85 to 90 percent (S_{sm}) level in the FAME classification. Despite the overall similarity between the two classifications, OTUs not recovered in groups in one classification were often recovered in groups in the other. This indicates at least a partial lack of congruence between the FAME and phenetic numerical classifications.

OTUs recovered in a phenetic group would often split into two or more FAME groups. In other cases OTUs recovered in different phenetic groups would be recovered in a single FAME group. In most phenetic groups (67 percent) some degree of splitting occurred. Splitting occurred in all larger groups.

It was previously suggested (Part II) that the phenetic groupings are direct estimates of the fundamental niche of the OTUs recovered in that group. It also was suggested that recovery of OTUs is dependent on their concentration in the environment, potential ability to grow on the isolation medium, and physiological state in the environment. The first two factors have been well studied, however, the third factor has been somewhat neglected. The concept of dormancy has been brought up by Stevenson (103), and evidence exists, both in terms of activity and isolation, that many aquatic bacterial populations are stressed and are perhaps dormant (29, 30, 34, 43, 46). Thus, it can be hypothesized

that populations isolated from the stream sediments estimate fundamental niches in which the OTUs were present in appreciable numbers, preadapted, to some extent, to the isolation medium, and were physiologically fit. Each fundamental niche (phenetic group) may represent one or more realized niches. Taxonomically, species are phenetically defined at S_{sm} similarity levels identical to, or only slightly higher than those used in this study (40, 48, 82, 108). Genera are normally defined at lower similarity levels (40, 108). Ecological theory holds that one species will occupy one realized niche given a stable environment (45, 52). Therefore, the number of realized niches per estimated fundamental niche in this investigation would tend to be low. In the shallow aerobic portion of the sediment, environmental parameters fluctuate thus changing the potential roles available to the bacteria. The distribution of OTUs defined in the phenetic groups (fundamental niches) in the FAME groups suggests that, although phenetically well defined, a significant amount of taxonomic variation exists within the fundamental niche. It should be noted, however, that when phenetic groups split, with the exceptions of groups 3 and 12, in the FAME defined groups, OTUs from a phenetic group would be recovered in FAME groups that shared high intergroup similarities, e.g. group 8 split into FAME groups D, E and F.

The OTUs recovered in FAME defined groups were distributed in phenetically defined groups in much the same manner as the OTUs from phenetically defined groups were distributed in FAME groups. However, the dispersion of OTUs into dissimilar groups was greater. Four groups, A, C, D and I were recovered in highly diverse phenetic groups as measured using intergroup similarities. These results are somewhat

surprising given the high level of similarity used to define the FAME groups. If the FAME classification had been made at too fine a level (high similarity), it would be expected that OTUs from one FAME defined group would be recovered in only one phenetic group, or that OTUs from two or more FAME defined groups would be recovered in one phenetic group. Two opposing conclusions can be drawn from these results: (i) the FAME classification is not highly split, or (ii) the FAME classification is poor. The second conclusion is unlikely due to the conservative nature of the data coding, the error control, the range of similarities (discrimination) obtained for both the S_{sm} and S_J coefficients, and the congruence of the classifications obtained using the S_{sm} and S_J coefficients. The dispersion of the FAME classified OTUs into dissimilar phenetic groups may indicate the presence of chemotaxonomically (genetically) distinct taxa which have a wide range of physiological and biochemical (particularly potential carbon sources) potentials. A taxon of this sort would bear a strong resemblance to an "r" selected species (70) in that both being present in the community (in the form of dormant or resting states, or through growth, or through recruitment) and having a wide and varied fundamental niche may be of more importance than high efficiency and specializaion. A highly efficient and specialized species could be considered "K" selected and would be characterized by a narrow and constant fundamental niche (70).

V. SUMMARY

In this investigation there appear to be two major trends in the bacterial guild stucture. One major trend is that phenetic groups, defined at similarity levels associated with subgeneric or specific taxa and taken as estimates of fundamental niches, appear to be somewhat diverse when examined chemotaxonomically. Lack of or incomplete competition between populations due to changing environmental factors (and therefore, changing potential roles) was suggested as a mechanism which allows for significant taxonomic variation. Chemotaxonomic variation was observed in 67 percent of the phenetic groups. Chemotaxonomic characterization was used in order to classify the OTUs in a manner which would operate independently of their fundamental niche in regards to carbon source utilization and other biochemical characters. A second major trend is that highly related (chemotaxonomically) OTUs often were recovered in diverse phenetic groups. The presence of taxonomic groups with large and highly variable biochemical potential, "r" selected populations, was suggested as an explanation of the dispersion of OTUs in FAME defined groups into highly dissimilar phenetic groups.

Neither of the above two trends is thought to operate independently of each other. Rather, both selection over time to form "r" selected species and short term adaption of diverse populations to the changing potential roles in the environment represent two overlapping factors allowing for significant bacterial activity and growth, and therefore, energy flow and nutrient cycling in a constantly changing environment.

LIST OF REFERENCES

LITURATURE CITED

1. Agbo, J. A. C. and M. O. Moss. 1979. The isolation and characterization of agarolytic bacteria from a lowland river. J. Gen. Microbiol. 115:355-368.

2. Allison, M. J., I. M. Robinson, J. A. Bucklin and G. D. Booth. 1979. Comparison of bacterial populations of the pig cecum and colon based upon enumeration with specific energy sources. Appl. Environ. Microbiol. 37:1142-1151.

3. Austin, B., M. Goodfellow and C. H. Dickinson. 1978. Numerical taxonomy of phyloplane bacteria isolated from Lolium peranne J. Gen. Microbiol. 104:139-155.

4. Austin, M. P. and L. Orloce. 1966. Geometric models in ecology II. An evaluation of some ordination techniques. J. Ecol. 54:217-227.

5. Baas-Decking, L. G. M., I. R. Kaplan and D. Moore. 1960. Limits of the natural environment in terms of pH and oxidation-reducition potential. J. Geol. 68:243-284.

6. Bailey, R. W. and E. G. Scott. 1974. Diagnostic Microbiology. The C. V. Mosby Co., Saint Louis, MO.

7. Baleux, B. 1977. A compter study of the evolution of aerobic heterotrophic bacterial populations in sewage and river waters. Microbial Ecol. 4:53-65.

8. Barn, A. J., J. H. Goodnight, J. P. Sall and J. T. Helwig. 1976. A users guide to SAS 76. Sparks Press, Raleigh, N. C.

9. Baumann, P., M. Doudoroff and R. Y. Stanier. 1968. A study of Naumella group II. Oxidase-negative species (genus Acenetobacter). J. Bacteriol. 95:1520-1541.

10. Berland, B.R., D. J. Bonin, J. -P. Durbee et S. Y. Maestrini. 1979. Bacteries heterotropes aerobes prelevees devant le delta lu Rhone I - Estimation des populations et determination. Hydrobiologia 117:481-497.

11. Blakemore, R. P. and A. E. Carey. 1978. Effects of poly-chlorinated biphenyls on growth and respiration of heterotophic marine bacteria. Appl. Environ. Microbiol. 35:323-328

12. Bobbie, R. J., S. J. Morrison and D. C. White. 1978. Effects of substrate biodegradability on the mass and activity of the associated estuarine microbiota. Appl. Environ. Microbiol. 35:179-184

13. Bobbie, R. J. and D. C. White. 1980. Characterization of benthic microbial community structure by high-resolution gas chromatography of fatty acid methyl esters. Appl. Environ. Microbiol. 39:1212-1222.

14. Bøe, B. and J. Gjerde. 1980. Fatty acid patterns in the classafication of some representatives of the families Enterobacteriaceae and Vibrionaceae. J. Gen. Microbiol. 116:41-49.

15. Bohme, B. 1906. Methyl rote prüfung. Central Blatt für Bacteriologie Orig. 40:129-134.

16. Brock, T. D. 1966. Principles of Microbial Ecology. Prentice-Hall Inc. Englewood Cliffs, N. J.

17. Buchanan, R. E. and N. E. Gibbons. 1974. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, MD.

18. Buck, J. D. 1979. The plate count in aquatic microbiology. in Aquatic Bacteria: Enumeration, Activity, and Ecology. J. W. Costerton and R. R. Colwell, eds. American Society for Testing and Materials. pp. 19-28.

19. Clark, K. and L. Lubs. 1915. Significance of acetoin production. J. Infect. Dis. 17:160-164.

20. Coblentz, L. M. 1943. Rapid detection of the production of acetyl-methyl-carbinol. Amer. J. Pub. Health 33:815-817.

21. Colwell, R. R. 1970. Polyphasic taxonomy of the genus Vibrio: numerical taxonomu of Vibrio cholerae, Vibrio paraheamolyticus, and related Vibrio species. J. Bacteriol. 104:410-433.

22. Colwell, R. R. and W. J. Weibe. 1970. "Core" characteristics for use in classifying aerobic, heterotrophic bacteria by numerical taxonomy. Bull. Ga. Acad. Sci. 28:165-185.

23. Cowen, S. T. 1955. Introduction to the philosophy of classification. J. Gen. Microbiol. 12:314-319.

24. Cowen, S. T. 1970. Heretical taxonomy for bacteriologists. J. Gen. Microbiol. 61:145-154.

25. Cowen, S. T. 1971. Sense and nonsense in bacterial taxonomy. J. Gen. Microbiol. 67:1-8

26. Cowen, S. T. and K. J. Steel. 1965 Manual for the Identification Medical Bacteria. University Press, Cambridge.

27. Cranwell, P. A. 1979. Decomposition of aquatic biota and sediment formation: bound lipids in algal detritus and lake sediments. Freshwater Biol. 9:305-313.

28. Daley, R. J. 1979. Direct epifluorescence enumeration of native aquatic bacteria: uses, limitations, and comparative accuracy. in Native Aquatic Bacteria: Enumeration, Activity and Ecology. J. W. Costerton and R. R. Colwell, eds. American Society for Testing and Materials. pp. 29-45.

29. Dawe, L. L. and W. R. Penrose. 1978. "Bactericidal" property of seawater: death or debilitation? Appl. Environ. Microbiol. 35:829-833.

30. Dietz, A. S., L. J. Albright and T. Tuominen. 1976. Heterotrophic activity of bacterioneustun and bacterioplankton. Can. J. Microbiol. 22:1699-1709.

31. DiGeronimo, M. J., M. Nikaido and M. Alexander. 1978. Most-probable-number technique for the enumeration of aromatic degraders in natural environments. Microbial Ecol. 4:263-266.

32. Drucker, D. B. 1974. Chemotaxonomic fatty-acid fingerprints of some streptococci with subsequent statistical analysis. Can. J. Microbiol. 20:1723-1727.

33. Elton, C. 1927. Animal Ecology. Sidgwick and Jackson, London pp. 63-68.

34. Faust, M. A. and D. L. Correll. 1977. Autoradiographic study to detect metabolically active phytoplanktun and bacteria in the Rhode River Estuary. Mar. Biol. 41:293-305.

35. Fox, G. E., E. Stackdbrandt, R. B. Hespell, J. B. Gibson, J. Maniloff, T. A. Dyer, R. S. Wolfe, W. E. Balch, R. S. Tanner, L. J. Magrun, L. B. Zablen, R. Balkemore, R. Gupta, L. Bonen, B. J. Lewis, D. A. Stahl, K. R. Luehrsen, K. N. Chen and C. R. Woese. 1980. The phylogeny of procaryotes. Science 209:457-463.

36. Gause, G. F. 1934. The Struggle for Exsistence. Williams and Wilkins, Baltimore, MD.

37. Gee, J.M., B. M. Lind, G. Metcalf and J. L. Peel. 1980. Properties of a new group of alkalophilic bacteria. J. Gen. Microbiol. 117:9-17.

38. Goldfine, H. 1972. Comparative aspects of bacterial lipids. in Advances in Microbial Physiology. A. H. Rose and D. W. Tempest eds. Academic Press, New York, N. Y.

39. Goodfellow, M. 1971. Numerical taxonomy of some mocardioform bacteria. J. Gen. Microbiol. 69:33-80.

40. Goodfellow, M. and G. Alderson. 1977. The actinomycete-genus Rhodococcus: a home for the rhodochrous complex. J. Gen. Microbiol. 100:99-122.

41. Goodfellow, M., M. D. Collins and D. E. Minnikin. 1976. Thin-layer chromatographic analysis of mycolic acid and other long-chain components in whole organism methanolysates of coryneform and related taxa. J. Gen. Microbiol. 96:351-358.

42. Gordon, R. E. 1978. A species definition. Int. J. Syst. Bacteriol. 28:605-607.

43. Goulder, R., A. S. Blanchard, P. L. Sanderson and B. Wright. 1979. A note on the recognition of pollution stress in populations of estuarine bacteria. J. Appl. Bacteriol. 46:285-289.

44. Grimont, P. A. D., F. Grimont, H. L. C. Dulong de Rusnay and P. H. A. Sneath. 1977. Taxonomy of the genus Serratia. J. Gen. Microbiol. 98:39-66.

45. Grinnell, J. 1917. The niche-relationships of the California Trasher. Auk 34:427-433.

46. Harvey, R. W. S. and T. H. Price. 1977. Observations on pre-enrichment for isolating salmonellas from sewage polluted natural water using Muller-Kauffmann tetrathionate broth prepared from fresh and disiccated ox bile. J. Appl. Bacteriol. 43:145-148.

47. Hardin, G. 1960. The competitive exclusion principle. Science 131:1292-1297.

48. Hayes, P. R. 1977. A taxonomic study of Flavobacteria and related Gram negative yellow pigmented rods. J. Appl. Bacteriol. 43:345-367.

49. Holder-Franklin, M. A., M. Franklin, P. Cashion, C. Cornier and L. Wuest. 1978. Population shifts in heterorophic bacteria in a tributary of the Saint John River as measured by taxometrics. in Microbial Ecology. M. W. Loutit and J. A. R. Miles, eds. Springer-Verlag, New York, N. Y.

50. Hucker, G. J. and H. J. Cohn. 1923. Methods of Gram staining. Tech. Bull. N.Y. State Agr. Exp. Sta. no. 93.

51. Hugh, R. and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bacteriol. 66:24-26.

52. Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symp. Quant. Biol. 22:415-427

53. Hutchinson, G. E. 1961. The paradox of the plankton. Amer. Naturalist 95:137-145.

54. Jeffries, H. P. 1970. Seasonal composition of temperate phytoplankton communities: fatty acids. Limnol. Oceanogr. 15:419-426.

55. Jeffries, H. P. 1972. Fatty acid ecology of a tidal marsh. Limnol. Oceanogr. 17:433-440.

56. Jeffries, H. P. 1979. Biochemical correlates of seasonal change in marine communities. Amer. Naturalist 113:643-658.

57. Jones, J. G. 1980. Some differences in the microbiology of profundal and littoral lake sediments. J. Gen. Microbiol. 117:285-292.

58. Kaneda, T. 1977. Fatty acids of the genus Bacillus: an example of the branch chain preference. Bacteriol. Revs. 41:391-418.

59. Kaneko, T., R. M. Atlas and M. Krichersky. 1977. Diversity of bacterial populations in the Beaufort Sea. Nature, London 270:596-599.

60. Kaneko, T. and R. R. Colwell. 1978. The annual cycle of Vibrio parahaemolyticus in Chesapeake Bay. Microbial Ecol. 4:135-155.

61. Kaneko, T., J. Hauxhurst, M. Krechersky and R. R. Atlas. 1978. Numerical taxonomic studies of bacteria isolated from artic and subartic marine environments. in Microbial Ecology. M. W. Loutit and J. A. R. Miles, eds. Springer-Verlag, New York N. Y.

62. Kaneko, T., M. I. Krichersky and R. M. Atlas. Numerical taxonomy of bacteria from the Beaufort Sea. J. Gen. Microbiol. 110:111-125.

63. Kaplan, W. A., J. M. Teal and I. Valiela. 1977. Denitrification in salt marsh sediments: evidence for sasonal temperature selection among populations of denitrifies. Microbial Ecol. 3:193-204.

64. King, E. O., M. K. Ward and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.

65. King, J. D., D. C. White and C. W. Taylor. 1977. Use of lipid composition and metabolism to examine structure and activity of detrital microflora. Appl. Environ. Microbiol. 33:1177-1183.

66. Kovacs, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature, london 168:703

67. Larsen, H. 1962. Halophilism. in The Bacteria a Treatise on Structure and Function. Vol. 4. I. C. Gunsalus and R. Y. Stanier eds. Acacdemic Press, New York, N. Y.

68. Lechevalier, M. P. 1977 Lipids in bacterial taxonomy - a taxonomists view. CRC Crit. Rev. Microbiol. &:109-210.

69. Lovelace, T. E. and R. R. Colwell. 1968. A multipoint inoculator for petri dishes. Appl. Microbiol. 16:944:945.

70. McArthur, R. H. and Wilson, E. U. 1967. The theory of island biogeography. Princeton University Press, Princeton, N. J.

71. Mallory, L. M., B. Austinand R. R. Colwell. 1977. Numerical taxonomy and ecology of oligotrophic bacteria isolated from the estuarine environment. Can. J. Microbiol. 23:733-759.

72. Martin, V. P. and M. A. Bianchi. 1980. Structure, diversity and catabolic potentialities of aerobic heterotrophic bacterial populations associated with the continuous cultures of natural marine phytoplankton. Microbial Ecol. 5:265-279.

73. Michener, C. D. and R. R. Sokal. 1957. A quantitative approach to a problem in classification. Evolution 11:130-162.

74. Miller, G. A. 1969. A psychological method to investigate verbal concepts. J. Math Psychol. 6:169-191.

75. Moeller, V. 1955. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta. Pathol. Microbiol. Scand. 36:158-172.

76. Martin, W. J. and W. H. Ewing. 1967. The deoxy-ribonuclease test as applid to certain gram negative bacteria. Can. J. Microbiol. 13:616-618.

77. Mordarska, H., M. Mordarski and M. Goodfellow. 1972. Chemotaxonomic characters and classification of some nocardioform bacteria. J. Gen. Microbiol. 71:77-86.

78. Morita, R. Y. 1974. Temperature effects of marine microorganisms. in Effects of the Ocean Environment on Microbial Activities. R. R. Colwell and R. Y. Marita eds. University Park Press, Baltimore, MD.

79. Moss, C. W., M. A. Lambert and W. H. Merwin. 1974. Comparison of rapid methods for analysis of bacterial fatty acids. Appl. Microbiol. 28:80-85.

80. Nie, N. H., C. H. Hull, J. G. Jenkins, K. Stenbrenner and D. H. Bent. 1970. Statistical Package for the Social Sciences McGraw-Hill, New york, N. Y.

81. Nedwell, D. B. and G. D. Floodgate. 1971. The seasonal selection by temperature of heterotrophic bacteria in an intertidal sediment. Mar. Biol. 11:306-310

82. Pagel, J. E. and P. L. Seyfried. 1976. Numerical taxonomy of aquatic Acinetobacter isolates. J. Gen. Microbiol. 95:220-232.

83. Pedersen, D. P. and G. S. Sayler. 1980. Methanogenesis in freshwater sediments: inherent variability and effects of environmental contaminants. Can. J. Microbiol. (submitted for publication).

84. Pfister, R. M. and P. R. Burkholder. 1965. Numerical taxonomy of some bacteria isolated from antarctic and tropical seawater. J. Bacteriol. 90:863-872.

85. Popoff, M. and M. Vernon. 1976. A taxonomic study of the Aeromonas hydrophila - Aeromonas punctata group. J. Gen. Microbiol. 94:11-22.

86. Rokem, J. S., I. Goldberg and I. Mateles. 1980. Growth of mixed cultures of bacteria on methanol. J. Gen. Microbiol. 116:225-232.

87. Sayler, G. S. 1980. Impact of a coking effluent discharge on the microbial communities of freshwater sediments. Final report: Union Carbide Sub-contract 7182. Oak Ridge National Lab. Oak Ridge, TN.

88. Sayler, G. S., L. C. Lund, M. P. Shiaris, T. W. Sherrill and R. E. Perkins. 1979. Comparative effects of Aroclor 1254 (polychlorinated biphenyls) and phenanthrene on glucose uptake by freshwater microbial populations. Appl. Environ. Microbiol. 37:878-885.

89. Shaw, N. 1974. Lipid composition as a guide to the classification of bacteria. Adv. Appl. Microbiol. 17:63-108.

90. Shannon, C. E. and W. Weaver. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, IL.

91. Sherrill, T. W. and G. S. Sayler. 1980. Phenanthrene biodegradation in freshwater environments. Appl. Environ. Microbiol. 39:172-178.

92. Shiaris, M. P. and G. S. Sayler. 1980. Microbial degradation of polychlorinated biphenyls (PCB) in a freshwater environment. Appl. Environ. Microbiol. (submitted for publication)

93. Sieburth, J. McN. 1967. Seasonal selection of estuarine bacteria by water temperature. J. Exp. Mar. Biol. Ecol. 1:98-121.

94. Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Anton. van Leeuw. 23:15-22.

95. Simidu, U., E. Kaneko and N. Taga. 1977. Microbiological studies of Tokyo Bay. Microbial Ecol. 3:173-191.

96. Sneath, P. H. A. 1957. Some thoughts on bacterial classification. J. Gen. Microbiol. 17:184-200.

97. Sneath, P. H. A. 1957. The application of computers to taxonomy. J. Gen. Microbiol. 17:201-226.

98. Sneath, P. H. A. 1974. Test reproducibility on relation to identification. Int. J. Sys. Bacteriol. 24:508-523.

99. Sneath, P. H. A. and R. Johnson. 1972. The influence on numerical taxonomic imilarities of errors in microbiological tests. J. Gen. Microbiol. 72:377-392.

100. Sneath, P. H. A. and R. R. Sokal. 1973. Numerical Taxonomy. W. H. Freeman and Company, San Francisco, CA.

101. Sokal, R. R. and C. D. Michener. 1958. A statistical method for evaluating systematic relationships. University of Kans. Sci. Bull. 38:1419-1438.

102. Stevenson, I. L. 1967. Utilizaition of aromatic hydrocarbons by Arthrobacter spp. Can. J. Microbiol. 13:205-211.

103. Stevenson, L. H. 1978. A case for bacterial dormancy in aquatic systems. Microbial Ecol. 4:127-133.

104. Stanier, R. Y., E. A. Adelberg and J. L. Ingraham. 1976. The Microbial World. Prentice-Hall, Englewood Cliffs, N. J.

105. Tsukamura, M. 1974. A further nimerical taxonomic study of the rhodochrous group. Jap. J. Microbiol. 18:37-44.

106. Veldkamp, H. 1977. Ecological studies with the chemostat. in Microbial Ecology, Vol. 1. M. Alexander ed. Plenum Press New York, N. Y.

107. White, D. C., W. M. Davis, J. S. Nickels, J. D. King and R. J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oceologia. Berlin 40:51-62.

108. Wilkinson, B. J. and D. Jones. 1977. A numerical taxonomic survey of Listeria and related bacteria. J. Gen. Microbiol. 98:399-421.

109. Williams, W. T., G. N. Lance, L. J. Webb, J. G. Tracey and J. H. Connell. 1969. Studies in the numerical analysis of complex rain-forest communities IV. a method for the elucidation of small-scale forest pattern. J. Ecol. 57:635-654.

110. Wishart, D. and L. Leach. 1970. A multivariate analysis of Platonic prose rhythm. Computer Stud. Humaniteds Verbal Behav. 3:90-99.

111. ZoBell, C. E. and R. Y. Morita. 1957. Barophilic bacteria in some deep sea sediments. J. Bacteriol. 73:563-568.

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