A NUMERICAL TAXONOMIC STUDY OF COLD OCEAN MARINE BACTERIA FROM THE NORTHWEST ATLANTIC NEAR NEWFOUNDLAND

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A Numerical Taxonomic Study Of Cold Ocean Marine Bacteria From The Northwest Atlantic Near Newfoundland

by

© Brendan Thomas Hollohan, B.Sc. (Hons.)

A Thesis, submitted in partial
fulfillment of the requirements for
the degree of Master of Science

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ABSTRACT

One hundred and forty bacterial strains were isolated from two sources, giant scallop (Placopecten magellanicus) and seaweed (Alaria esculenta). The isolations from the seaweed were made on three successive occasions during the natural degradation of the fronds. Nine type strains from the genera Vibrio, Pseudomonas, Alteromonas and Photobacterium were also included. Approximately 150 characterization tests were scored for each strain. All of the cold ocean strains were gram-negative, motile rods and required Na^+ for growth. Approximately 60% were fermentative. The results of 112 tests were explored using cluster analysis and a variety of methods yielded six robust clusters. These clusters conformed largely to source of strains. The strains from A. esculenta clustered separately from those isolated from P. magellanicus. To some extent the clusters could be further distinguished as containing either oxidative or fermentative strains and, in the case of the seaweed isolates, by time of isolation, i.e., early or later in the degradation process. Tests which discriminated each cluster were identified. The predominant bacteria isolated were from the genera Vibrio, Pseudomonas and Alteromonas. Although some of the strains could be identified at the species level others could not. The latter strains may represent new species of marine bacteria.

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"..... the sure and definite determination (of species of bacteria) requires so much time, so much acumen of eye and judgement, so much of perseverance and patience that there is hardly anything else so difficult". - Mueller.

INTRODUCTION

The early work

Bacteria isolated from marine sources have been objects of scientific investigation for approximately a century. The first comprehensive study of bacteria indigenous to oceans was performed by Fischer while on the plankton expedition of the Humboldt Foundation to the West Indies (Fischer, 1894). Fischer did extensive bacterial counts of surface waters of the north Atlantic Ocean from southern Greenland to the Caribbean Sea and of the south Atlantic Ocean to Ascension Island. Fischer described the cultural characteristics and named some of the bacteria isolated (Fischer, 1894 and cited from ZoBell, 1946). Luminescent bacteria were placed in the genus Photobacterium Beijerinck 1889 and several non-luminescent heterotrophic bacteria were assigned to a newly created genus Halibacterium Fischer 1894. The Halibacterium strains required a medium prepared with seawater for optimum growth. The prefix "hali" denoted salt or sea.

A comprehensive review of the early literature in marine bacteriology was published by ZoBell and Upham (1944). This publication lists the marine bacteria known at the time and includes descriptions of 60 new species. Predominant among the heterotrophic bacteria isolated by 1944 were genera belonging to several of the groups that now make up the 18 parts of the 8th edition of

Bergey's Manual (Buchanan and Gibbons, 1974). Some of these are: the actinomycetes Actinomyces Harz 1877, Micromonospora Ørskov 1923, Mycobacterium Lehmann and Neumann 1896, and Nocardia Trevisan 1889; the gliding bacteria Achromatium Scheiwiahoff 1893, Beggiatoa Trevisan 1842, Saprospira Gross 1911, and Thiothrix Winogradsky 1888; the spirochete Cristispira Gross 1910; the spiral-shaped Spirillum Ehrenberg 1832; the gram-positive coccus Micrococcus Cohn 1872; the gram-positive endospore-forming Bacillus Cohn 1872; the gram-negative aerobic rods Agrobacterium Conn 1942 and Pseudomonas Migula 1894; the anaerobic Clostridium Prazmowski 1880; and finally the gram-negative facultatively anaerobic genera Chromobacterium Bergonzini 1881, Flavobacterium Bergey et al. 1923, Serratia Bizio 1823, Photobacterium and Vibrio Pacini 1854.

There were no determinative schemes specific for marine bacteria at this time and, at the time of ZoBell and Upham's (1944) review of the literature the current edition of Bergey's Manual of Determinative Bacteriology was the 5th (Bergey et al., 1939). Of 1,335 bacteria described in the 5th edition only 86 had been isolated from the sea. Therefore, it is evident that marine bacteriology had not received a great deal of attention in the early years of bacteriology. In part, this may have been because, at that time, there were no known pathogenic marine bacteria.

Classification by the determinative schemes of Shewan and coworkers.

A significant advance in systematic aquatic bacteriology was made when Shewan et al. (1960) published a scheme for the identification of certain genera of aerobic and facultatively anaerobic, heterotrophic, gram-negative bacteria, especially the Pseudomonadaceae Winslow et al. 1917. Based on relatively few bacteriological tests this scheme had been developed to facilitate the identification of isolates from the marine environment and from spoiling fish. The scheme arranged these bacteria into four broad groups on the basis of motility and flagella, oxidase reaction (Kovacs, 1956) and pigment production: a) motile with polar flagella, oxidase positive (Pseudomonas, Aeromonas Kluyver and van Niel 1936, and Vibrio); b) motile with peritrichous flagella, oxidase negative (Enterobacteriaceae Rahn 1937); c) non-motile, non-pigmented short stout rods (Achromobacter Bergey et al. 1923 and Alcaligenes Castellani and Chambers 1919); and d) non-motile with yellow-pigmented colonies (Flavobacterium and Cytophaga Winogradsky 1929). Pseudomonas was distinguished from Vibrio and Aeromonas by reaction in O-F medium (Hugh and Leifson, 1953). Pseudomonas was subdivided into four groups by the presence or absence of diffusable pigment when grown on the medium of King et al. (1954) and also by the type of reaction in O-F medium. Aeromonas and Vibrio could

be distinguished by the production of gas from glucose and sensitivity to 0/129 (2,4-diamino-6,7-diisopropyl pteridine) (Shewan et al., 1954).

The original scheme was modified by Hendrie et al. (1964) such that oxidase positive, motile and peritrichously flagellated rods that were non-fermentative were either Achromobacter or Alcaligenes. In a more recent study Davis et al. (1969) proposed that all nonfermentative heterotrophic gram-negative rods with peritrichous flagella be placed in the single genus Alcaligenes, while Achromobacter be reserved for similar but non-motile organisms.

At the time the original scheme was published Shewan et al. (1960) stated that theirs was a convenient identification scheme that did not express a phylogenetic relationship between the groups identified. However, they had applied Adansonian principles in their study and the schemes presented were derived from data that had been numerically analyzed. Their determinative scheme was one of the first in bacteriology to be based on Adansonian principles. The scheme has proven to be versatile and is widespread in its use. Gibson et al. (1977) and Sochard et al. (1979) have presented useful revisions of the earlier scheme. The most recent revisions done by the original group are that of Hendrie and Shewan (1979) and Lee et al. (1979).

Ecological studies that have utilized the above schemes, and will be discussed later, are: Colwell (1962),

Colwell and Liston (1960, 1962a, 1962b), Beeson and Johnson (1967), Doores and Cook (1976), Simidu et al. (1971, 1977), Unkles (1977), Sochard et al. (1979), Madri et al. (1971), Reiswig (1975), Imhoff and Trüper (1976), Wilkinson (1978a, 1978b, 1978c), Atlas et al. (1982), Boyle and Mitchell (1981), Chan and McManus (1969), Laycock (1974), Kong and Chan (1979), Shiba and Taga (1980), Pfister and Burkholder (1965), Kaneko and Colwell (1973), Gauthier et al. (1975), Austin et al. (1979), Kaneko et al. (1979) and Hauxhurst et al. (1980). The widespread use of schemes based on that of Shewan et al. (1960) is evident from the above list.

Significance of the growth requirement for sodium ion by heterotrophic gram-negative marine bacteria.

Determinative schemes up to and including that of Shewan et al. (1960) do not differentiate between bacteria of marine and terrestrial origin although differences were known. Fischer (1894) observed that marine bacteria required seawater in the medium for optimum growth. ZoBell and Upham (1944) observed that, besides the specific salinity requirement of marine bacteria, they were not morphologically or physiologically distinguishable from terrigenous bacteria. These authors defined marine species as those which, upon initial isolation, grew in nutrient seawater media but not in corresponding freshwater media.

The role of inorganic ions in the growth and metabolism of marine bacteria was not more fully understood until approximately 25 years later (MacLeod, 1965, 1968). MacLeod and Onofrey (1956, 1957) found that seawater supplied the inorganic ions required for the growth of marine isolates. In particular, the requirement for Na^+ could not be replaced by related inorganic ions or by organic compounds added to increase the osmotic pressure of the medium. MacLeod and Onofrey (1956, 1957) postulated that gram-negative marine bacteria could be distinguished from terrestrial forms by having a readily detectable need for Na^+ in the medium for growth. This definition has become a useful taxonomic criterion for distinguishing gram-negative marine bacteria from their terrestrial and freshwater counterparts (Baumann and Baumann, 1981; Gow et al., 1981).

It should be acknowledged that MacLeod and Onofrey (1956, 1957) were not the first authors to describe the Na^+ requirement of marine bacteria. Richter (1928) showed that marine luminescent bacteria required Na^+ and that the Na^+ requirement could be easily overlooked if the organisms were grown on complex media contaminated with inorganic ions. Richter's (1928) publication remained relatively unknown until it was cited by MacLeod (1968). MacLeod and Onofrey (1956, 1957) were unaware of Richter's

(1928) publication at the time they initiated their own investigation into the inorganic ion requirements of marine bacteria.

The function of inorganic ions, especially Na^+, in the gram-negative marine bacteria has been recently reviewed by Pratt (1974) and Baumann and Baumann (1981).

Classification by the determinative schemes of Baumann and coworkers.

- A departure from the determinative schemes based on that of Shewan et al. (1960) has been made by Baumann et al. (1971a) and Baumann et al. (1972). To avoid confusion it should be noted that there are two Baumanns in this group. On some publications Paul Baumann is the first author while on other publications Linda Baumann is the first author. These authors are the first bacterial taxonomists to recognize marine bacteria as entities separate from their freshwater and terrestrial counterparts on the basis of a requirement by the marine strains for readily detectable quantities of Na^+ for growth.

(a) Characterization of the facultatively anaerobic heterotrophic marine bacteria.

Baumann et al. (1971a) studied 145 gram-negative, facultatively anaerobic, straight or curved rod-shaped bacteria of marine origin. By the scheme of Shewan et al. (1960) these organisms would have belonged to the genera Vibrio or Aeromonas. Baumann et al. (1971a) established

eight groups by numerical analysis. They were separable by multiple, unrelated phenotypic traits. Six groups which had deoxyribonucleic acid (DNA) containing 45 to 48 moles percent guanine plus cytosine (G + C) were assigned to a redefined genus Beneckea Campbell 1957. All of the strains in this genus, when grown in broth, had a single, polar flagellum. When grown on solid medium, many strains had peritrichous flagella. Two groups were similar to species already described, Oceanomonas alginolytica Miyamoto et al. 1961 and Pseudomonas natriegens Payne et al. 1961, and were designated B. alginolytica (Miyamoto et al. 1961) comb. nov. Baumann et al. 1971 and B. natriegens (Payne et al. 1961) comb. nov. Baumann et al. 1971, respectively. The remaining four groups were designated new species in the genus Beneckea. They were B. campbellii Baumann et al. 1971, B. neptuna Baumann et al. 1971, B. nereida Baumann et al. 1971, and B. pelagia Baumann et al. 1971. A group of phenotypically similar strains having many of the properties of the genus Beneckea had been excluded from the numerical analysis. These strains were readily separable from species of this genus and were designated B. parahaemolytica Baumann et al. 1971. Of the remaining groups, one was identified as Photobacterium fischeri Beijerinck 1889. The other group (referred to as B-2) which had about 41 moles % G + C content in its DNA could not be placed into existing genera.

During the past decade other species of Beneckea were described, including B. nigrapulchrituda Baumann et al. 1971 (Baumann et al., 1971b), B. harveyi Reichelt and Baumann 1973 (Reichelt and Baumann, 1973a), B. vulnifica Reichelt et al. 1979 and B. splendida I, II Reichelt et al. 1979 (Reichelt et al., 1976) B. proteolytica Baumann and Baumann 1976 (Baumann and Baumann, 1976), B. anguillarum I, II Baumann et al. 1978 (Baumann et al., 1978), and B. gazogenes Harwood et al. 1980 (Harwood, 1978).

Three new species of Photobacterium have been described: P. leiognathi Boisvert et al. 1967 (Reichelt and Baumann, 1975), P. angustum Reichelt et al. 1979 (Reichelt et al., 1976), and P. logei Harwood et al. 1980 (Bang et al., 1978).

Quite recently, however, as a result of studies on the amino acid sequence divergence of glutamine synthetase and superoxide dismutase (Baumann et al., 1980a), Baumann et al. (1980b) have suggested the abolition of the genus Beneckea and the assignment of its constituent species, along with Photobacterium fischeri and P. logei, to the genus Vibrio. The definitions of Vibrio and Photobacterium were modified accordingly.

In a review by Baumann and Baumann (1977) the authors point out that the genus Vibrio, as currently defined, consists of two ecologically distinct groups. One group is straight or curved rods of marine origin. The

other group is V. cholerae Pacini 1854 and related strains which are inhabitants of fresh water and the human intestine. The two groups are different because vibrios belonging to the former group require high concentrations of Na^+ for growth. The genus Aeromonas consists of oxidase positive, facultatively anaerobic, polarly flagellated or nonmotile straight rods which ferment glucose with or without the production of gas. Species of Aeromonas are common inhabitants of fresh water. In the original scheme of Shewan et al. (1960), strains of Aeromonas were distinguished from strains of Vibrio by the insensitivity of the former genus to the vibriostatic pteridine compound (0/129). However, this test is not reliable for marine bacteria in view of the findings of Merkel (1972) who has shown that salts in seawater medium may inhibit the vibriostatic action of the pteridine compound. The sole reliable criterion for differentiation between Aeromonas and Vibrio is a molecular one. Members of the genus Vibrio have a base ratio of 40 to 50 moles % G + C in their DNA, whereas members of the genus Aeromonas have 50 to 60 moles % G + C (Sochard et al., 1979). However, this test is difficult to perform in routine bacteriological studies. According to Baumann and Baumann (1977) the genus Aeromonas would consist of strains that were of freshwater origin and presumably would not require Na^+ for growth. They suggested that the facultatively anaerobic bacteria requiring Na^+ for growth be assigned to the genus Beneckea (later changed

to Vibrio). These generic separations have been supported by the results of DNA/RNA homology studies (Baumann and Baumann, 1976).

This recommendation received official approval in 1980 when the Ad Hoc Committee of the Judicial Commission of the International Committee on Systematic Bacteriology (I.C.S.B.) published its approved lists of bacterial names (Ad Hoc Committee, 1980). Nine species of Beneckea (later changed to Vibrio) were listed and V. campbellii was designated the type species. V. nereis and V. gazogenes were published as valid bacterial names by Harwood et al. (1980). The name V. vulnificus was revived by Farmer (1980).

Jensen et al. (1980a) submitted 22 facultatively anaerobic marine bacteria belonging to Group F of Lee et al. (1978) to an extensive phenotypic characterization. The results of a numerical analysis of the data indicated that this group consisted of two biotypes which were phenotypically distinct from 25 previously characterized species of Vibrio and Photobacterium. The Group F strains were later described and named as a new species, Vibrio fluvialis Lee et al. 1981 (Lee et al., 1981).

(b) Characterization of the aerobic heterotrophic marine bacteria.

Baumann et al. (1972) characterized 218 strains of aerobic heterotrophic marine bacteria. All the strains were gram-negative, straight or curved rods which were

motile by means of polar or peritrichous flagella, and required Na^+ for growth. Computer analysis of the results of the physiological and nutritional characterization clustered the strains into 22 groups on the basis of phenotypic similarities. Analysis of the moles % G + C in the DNA of representative strains indicated that the peritrichously flagellated groups had a G + C content of 53.7 to 67.8 moles percent; polarly flagellated strains had a G + C content of 30.5 to 64.7 moles percent. The peritrichously flagellated groups were assigned to the genus Alcaligenes, and four new species were named: A. pacificus Baumann et al. 1972, A. cupidus Baumann et al. 1972, A. venustus Baumann et al. 1972, and A. aestus Baumann et al. 1972. The polarly flagellated groups which had a G + C content of 43.2 to 48.0 moles percent were placed into a newly created genus Alteromonas Baumann et al. 1972. Four new species were described; A. communis Baumann et al. 1972, A. vaga Baumann et al. 1972, A. macleodii Baumann et al. 1972, and A. marinopraesens Baumann et al. 1972. The polarly flagellated groups which had a G + C content of 57.8 to 64.7 moles percent were placed into the genus Pseudomonas, and three new species were named: P. doudoroffii Baumann et al. 1972, P. marina Baumann et al. 1972, and P. nautica Baumann et al. 1972. The remaining 10 groups were left unassigned.

It was later discovered that the naming of Alteromonas marinopraesens was erroneous and its name was

changed to A. haloplanktis Reichelt and Baumann 1973 (Reichelt and Baumann, 1973b). The genus Alteromonas received official nomenclatural validity and A. macleodii was designated the type species (Ad Hoc Committee, 1980).

Baumann et al. (1972) recognized that, although the species and groups described in their study were well characterized, their generic assignments were in part cumbersome, because of the dependence on flagellation and moles % G + C for generic separations. They recommended that further work was necessary in order to obtain adequate generic traits which would allow the aerobic marine eubacteria to be placed in well-defined genera.

Since 1972, other authors have named new species of Alteromonas. These are: A. rubra Gauthier 1976 (Gauthier, 1976a), A. citrea Gauthier 1977 (Gauthier, 1977), A. espejiana Chan et al. 1978 and A. undina Chan et al. 1978 (Chan et al., 1978), and A. aurantia Gauthier and Breittmayer 1979 (Gauthier and Breittmayer, 1979). A. luteoviolacea Gauthier 1982 was isolated and described by Gauthier (1976b) and validly named by Gauthier (1982). A. hanedai Jensen et al. 1981 was isolated and described by Jensen et al. (1980b).

The work of these authors has aided in the development of a better generic definition of the genus Alteromonas, which appears to consist of two phenotypically distinct groups of closely related species (Baumann and Baumann, 1981). One group consists of A. communis and

A. vaga and the other group consists of A. macleodii (the type species of Alteromonas), A. luteoviolacea, A. haloplanktis, A. rubra, A. citrea, A. espejiana, A. andina and A. hanedai. The relationship between the two groups is still under study. Baumann and Baumann (1981) have suggested that the possible exclusion of A. communis and A. vaga may allow a better definition of Alteromonas.

In their review of the taxonomy of the marine eubacteria Baumann and Baumann (1981) state that, although the results of extensive studies on the fermentative marine bacteria permit generic assignments which are partially based on genetic relationships, studies of the oxidative marine bacteria are not as advanced. Consequently, it is necessary to make generic assignments within the latter group on the basis of flagellation and moles % G + C, and not on the basis of known natural relationships. For purposes of identification, the oxidative marine eubacteria are divided into three broad groups: a) Those which are polarly flagellated, rod-shaped organisms not accumulating poly-β-hydroxybutyrate (PHB). This category includes all species of Alteromonas (moles % G + C = 40-50), P. nautica (moles % G + C = 57-62), and a group designated H-2 (moles % G + C = 28-33). b) Polarly flagellated organisms which accumulate PHB, all of which are species and groups of Pseudomonas (moles % G + C = 54-64). c) Peritrichously flagellated organisms, which are assigned to the genus Alcaligenes.

Apart from studies which have resulted in the description of new bacterial species, the identification schemes of Baumann et al. (1971a) and Baumann et al. (1972) have received minimal usage over the past decade. Probably this is because these schemes are inherently more difficult to use than those derived from the original scheme of Shewan et al. (1960). The former schemes require a large number of characterization tests, including extensive nutritional screening of the isolates and the determination of moles % G + C. As a result, these schemes are laborious for ecological studies requiring the characterization of hundreds of isolates. However, with the acceptance of the genus Alteromonas and the recognition that other specific marine bacteria exist, the studies of Baumann's group cannot be ignored. Already an attempt has been made to integrate the determinative schemes reviewed by Baumann and Baumann (1981) with those which originate from Shewan et al. (1960). In schemes published by Hendrie and Shewan (1979) and Lee et al. (1979) the importance of the Na^+ requirement as a diagnostic characteristic is recognized and species of Alteromonas, Pseudomonas, Alcaligenes, Photobacterium and Vibrio can be differentiated.

Numerical taxonomy

The developments in bacterial taxonomy and in determinative schemes since 1960 are the result of the application of new approaches and techniques including

numerical taxonomy (Sneath, 1957a, 1957b), molecular biology and genetic analysis (Mandel, 1969). Indeed, the more precise and much more laborious method of numerical taxonomy has eclipsed the simpler approach (Sieburth, 1979).

Numerical taxonomy has been defined by Sneath and Sokal (1973) as "the grouping by numerical methods of taxonomic units into taxa on the basis of their character states". The principles of numerical taxonomy date back to the French botanist, Michel Adanson (early 17th century), and are frequently called neo-Adansonian. As stated by Colwell and Austin (1981) Adansonian principles are: "1) Maximum information content should be achieved, i.e., all possible tests should be studied for the strains. 2) The tests should be weighted equally. 3) Taxa should be defined on the basis of overall similarity according to the results of phenetic analyses (analysis based on the observed characters rather than on their ancestry)".

The principal aims of numerical taxonomy are repeatability and objectivity. "If observations are repeatable within an acceptable error and if taxonomic procedures are clearly circumscribed, it is hoped that numerical methods will lead different scientists employing the same data base and working independently to obtain comparable estimates of the resemblance among any group of organisms" (Sneath and Sokal, 1973). The development

and availability of electronic computers, capable of performing repeated operations with large sets of data, are necessary requirements for the advance of numerical taxonomic methods. Data from many sources, e.g., morphology, physiology, and biochemistry can be included in a taxonomic study. This data may be stored and made available to other workers.

A numerical taxonomic study of bacteria involves the following essential steps (Colwell and Austin, 1981): 1) Selection of strains 2) Selection of tests 3) Coding and arraying of test results 4) Computer analysis of the relationships between strains and the clustering of related strains 5) Presentation and interpretation of the results.

The members of the set of entities to be studied by numerical taxonomy are referred to as operational taxonomic units (OTU). In this study they are the individual bacterial strains. With the availability of appropriate computer programs and adequate resources, numerical methods permit the analysis of data collected for a large number of bacterial strains. These should be selected without bias in order to avoid subjectivity on the part of the investigator. It is also important to include reference strains which have been identified and bear a scientific name. These known strains should, if possible, include authentic type cultures (Colwell and Austin, 1981).

An integral part of a numerical taxonomic study is the selection of routine characterization tests that

represent a broad spectrum of the biological activities of the organisms. An optimum number of tests which may yield results that are statistically valid is considered to be in the range of 100 to 200 (Colwell, 1973; Colwell and Austin, 1981). It is important to standardize the treatment of all strains in a test set whenever possible.

Eventually, all results for a set of OTU must be converted to a numerical format. The simplest is the binary code where a positive response is coded as 1 and a negative response as 0. There can be a provision for noncomparable characters which are usually coded as 3 (Colwell and Austin, 1981).

The most commonly used term for techniques which seek to separate data into constituent groups is "cluster analysis" (Everitt, 1974). The object of a cluster analysis is to sort a sample of individuals under investigation into groups such that the degree of association is high between members of the same group and low between members of different groups. The computations allow a logical stepwise examination of the data, including a determination of the overall similarity between strains and a hierarchical ordering of the strains according to their similarity. Many cluster analysis computer programs are available (Wishart, 1978).

The results of a numerical taxonomic study may be presented by several means, the most common being tree

diagrams or dendrograms (Colwell and Austin, 1981). Inspection of dendrograms allows one to define clusters of similar strains and determine similarities between clusters. Clusters of strains can be further characterized by tabulating the frequency of occurrence of each character among members of the clusters. This leads to the compilation of identification tables based on selected discriminatory tests. Such identification tables are useful for the subsequent identification of new strains. The inclusion of type or reference strains in the study allows for the possible identification and naming of clusters. This may also be achieved by comparing selected features of the clusters with those characteristics already published in dichotomous keys and diagnostic tables. Clusters which cannot be equated with existing named taxa may represent new and undescribed taxa.

For this study, the "Clustan" package of cluster analysis programs was used. The analyses performed were several that were recommended by Wishart (1978) to test the robustness of the classification obtained for populations of less than 150 individuals. The simple matching and Jaccard association coefficients were used because they are familiar to the majority of bacterial taxonomists (Colwell and Austin, 1981).

Association coefficients are most commonly computed with two-state characters which are conveniently

coded as 0 or 1 (Sneath and Sokal, 1973). When character states are compared for each pair of OTU the outcome can be summarized in a conventional 2 x 2 frequency table such as the one shown here (modified from Sneath and Sokal, 1973):

		OTU A: 1	OTU A: 0
OTU B	1	a	b
	0	c	d

$$n = a + b + c + d$$

The upper left quadrant of the figure contains the number of characters coded 1 (positive) in both OTU, while the lower right quadrant contains the number of characters coded 0 (negative) for both OTU. The other two quadrants register the number of characters in which the two OTU disagree, being coded 1 for OTU A and 0 for OTU B, or the converse. n denotes the sum of the four frequencies, which equals the number of characters in the study. The following discussion will be limited to the coefficients used in this study.

The simple matching coefficient (Ssm) has been described by Sneath and Sokal (1973) as one of the oldest and simplest association coefficients used in numerical taxonomy. It was introduced to numerical taxonomy by Sokal and Michener (1958) and has been used repeatedly since. Using the above 2 x 2 frequency table, the simple matching

coefficient may be defined as follows:

$$Ssm = \frac{a + d}{a + b + c + d}$$

The Jaccard association coefficient (Sj) was first introduced to numerical taxonomy by Sneath (1957a). The Jaccard coefficient is defined as follows:

$$Sj = \frac{a}{a + b + c}$$

It has been described by Sneath and Sokal (1973) as the simplest of the coefficients in its class.

The simple matching coefficient considers a total match between OTU by including all of the positive and negative matches, and thereby it provides an estimation of overall similarity. The Jaccard coefficient does not include negative matches in the equation. The merits of both equations have been discussed at length by Sneath and Sokal (1973) and Sokal and Sneath (1963). However, it has been emphasized by Colwell and Austin (1981) that the simple matching coefficient reflects the presence of a large number of negative attributes between strains, thereby giving an erroneous indication of similarity. These authors suggested that data be examined using both the simple matching and Jaccard coefficients.

In addition to association (similarity) coefficients, numerical taxonomists often employ distance coefficients which measure the distance between OTU in a

space defined in various ways. The most familiar measure of distance is simple Euclidean distance in a character space of one or more dimensions (Sneath and Sokal, 1973). These authors also point out that distance coefficients are the converse of similarity coefficients. The former are, in fact, measures of dissimilarity.

Euclidean distance is an extension of pythagorean geometry to t dimensions, where t equals the number of characters. The distance (d) between OTU i and j is given as:

$$dij = \frac{b + c}{a + b + c + d}$$

The Euclidean distance measure is a metric, ie., it has the properties of Euclidean geometry. If X, Y and Z are OTU and d the Euclidean distance, then the following properties apply (Everitt, 1974):

1) $d(X,Y) \geq 0$. If $d(X,Y) = 0$ then $X = Y$.

2) $d(X,Y) = d(Y,X)$.

3) $d(X,Y) \leq d(X,Y) + d(Y,Z)$.

The third condition is the one which differentiates most between distance measures and similarity measures. It is referred to as the "metric inequality" or "triangular inequality".

Using the output of the similarity or distance computations, cluster analysis can be used to arrange the OTU in a hierarchical order, according to overall

similarities. These cluster analysis methods are classified into five types by Everitt (1974) as follows: 1) hierarchical techniques, 2) partitioning techniques, 3) density search techniques, 4) clumping techniques, and 5) other techniques which do not fall clearly into any of the four previous groups.

Hierarchical techniques may be subdivided into "agglomerative" methods, which proceed by a series of successive fusions of the N entities into groups, and "divisive" methods, which partition the set of N entities successively into finer groups (Everitt, 1974). The results of both agglomerative and divisive methods may be presented in the form of a dendrogram. For this study two agglomerative techniques were used. These are the single linkage (nearest neighbour) method and Ward's method.

The single linkage method was introduced to numerical taxonomy by Sneath (1957b). It may be used with either similarity measures or distance measures (Everitt, 1974). With this method, the data matrix is scanned to find the pair of OTU with the highest similarity (or lowest distance) value. The matrix is subsequently rescanned to find additional pairs of OTU with the next highest similarity value. Such a pair may be OTU joining the emerging cluster, or if one of the pair has already been allocated to an existing cluster, the other strain can also be added to the cluster. The scanning process is repeated until all the OTU have been transferred from the unsorted

similarity matrix to a cluster (Colwell and Austin, 1981). In other words, groups initially consisting of single individuals are fused according to the distance between their nearest neighbours, the groups with the smallest distance being fused. Each fusion decreases by one the number of groups. Hence, for this method, the distance between groups is defined as the distance between their closest members (Everitt, 1974). A lengthy, technical discussion of single linkage clustering has been published by Sneath and Sokal (1973).

Ward's clustering method was introduced to numerical taxonomy by Ward (1963), who proposed that at any stage of an analysis the loss of information which results from the grouping of individuals into clusters can be measured by the total sum of squared deviations of every point from the mean of the cluster to which it belongs. At each step in the analysis, union of every possible pair of clusters is considered and the two clusters whose fusion results in the minimum increase in the error sum of squares are combined (Everitt, 1974). Hence, this method is based on a measure of within-group versus among-group differences (Sneath and Sokal, 1973). It starts out with t separate OTU, grouping them successively into t-1, t-2, t-3, ..., 1 taxa, and computing at each stage the sum of the within group sum of squares. A detailed but comprehensible example of this method may be found in Everitt (1974).

In addition to hierarchical techniques, the cluster analyses used in this study also included a density search technique. If OTU are depicted as points in a multidimensional space, a natural concept of clustering suggests that there should be parts of the space in which the points are very dense, separated by parts of low density. Density search techniques are cluster analysis techniques which seek regions of high density (or modes) in the data (Everitt, 1974).

Several of these methods have their origins in single linkage cluster analysis and arose in an attempt to overcome the main problem of that technique, namely "chaining". Chaining refers to the tendency of a technique to incorporate OTU into existing clusters rather than to initiate new clusters (Everitt, 1974).

The density search technique used in this study is similar to mode analysis. It was introduced to numerical taxonomy by Wishart (1969) and is a derivative of single linkage clustering which searches for natural sub-groupings of the data by estimating disjoint density surfaces in the sample distribution. The search is made by considering a sphere of some radius R surrounding each point (OTU), and counting the number of points falling in this sphere. Those individuals whose spheres contain K or more other points are called dense points. The remaining individuals whose spheres contain less than K other points are termed

non-dense (Everitt, 1974). At each cycle of the program the radius R is gradually increased so that the inclusion of more points is possible. The possible strategies considered with the introduction of each new point and the advantages of density analysis are described in detail in Wishart (1978).

The cluster analysis strategy used in this study also included a partitioning technique known as "relocate" (Wishart, 1978). Most partitioning techniques employ three distinct procedures (Everitt, 1974): 1) a method of initiating clusters, 2) a method for allocating entities to initiated clusters, and 3) a method of reallocating some or all of the entities to other clusters once the initial classificatory process has been completed.

Starting with a classification of the population of N objects into k clusters, during one relocation scan each object is considered in turn and its similarities with all k clusters are computed. Suppose that the similarity between object X and its parent cluster P is S (P,X) and the similarity between X and any other cluster Q is S (Q, X). Then if S (Q,X) exceeds S (P,X) the method moves X from cluster P to cluster Q. The population is repeatedly scanned until no objects are relocated during one full scan (Wishart, 1978). Detailed discussions of relocate methodology may be found in Everitt (1974) and Wishart (1978).

In summary, the numerical taxonomic strategy employed in this study consisted of obtaining initial classifications of the OTU using the simple matching association coefficient with single linkage clustering, the Jaccard association coefficient with single linkage clustering, and the Euclidean distance coefficient with Ward's clustering. Once the initial classification had been found, the data were rescanned to find clusters of high density (natural clusters) using the Jaccard association coefficient with density clustering, and then rescanned to search for OTU which should be relocated to other clusters using the Euclidean distance coefficient with relocate clustering. If the classifications produced by these methods coincide, then the classification is considered to be robust (Wishart, 1978).

Rationale for the study described in this thesis

In this study the taxonomic criterion described by Baumann and Baumann (1981) have been applied to marine bacteria isolated from the coastal waters of Newfoundland. Baumann et al. (1971a) and Bauman et al. (1972) studied and described marine mesophilic bacteria isolated from a number of sources. Included in their studies were named strains from existing culture collections. On the basis of their taxonomic studies some of these strains were placed in newly defined genera. In the study described in this thesis psychrophilic marine bacteria from two sources

were chosen and type cultures were included for reference only.

The sources of the strains were giant scallops (Placopecten magellanicus Gmelin) and the seaweed Alaria esculenta Greville. The strains were partially characterized in earlier studies. Powell (1978) isolated the strains from scallops and identified them using a limited number of tests described by Hendrie and Shewan (1966) and Bain and Shewan (1968). Eighty-three percent of the strains required Na^+ for growth and were defined as marine eubacteria. Hollohan (1980) isolated the strains from A. esculenta on three successive occasions during the process of degradation of the fronds. These strains were also identified using a limited number of tests, but this time the dichotomous key of Sochard et al. (1979) was used. Ninety percent of the strains from seaweed required Na^+ for growth.

Only strains requiring Na^+ for growth are included in this study. Powell (1978) described 80 and Hollohan (1980) described 108 Na^+ - requiring eubacteria. Because some of the clustering methods used were designed for populations of 150 OTU or less, not all of the strains originally described were included in this study. Approximately 70% of the OTU from each source were selected.

Many of the species of marine eubacteria that have been described are mesophilic (Baumann et al. 1971a; Baumann et al., 1972; Gauthier et al., 1975). Lee et al.

(1977) studied 14 strains of marine Pseudomonas-like bacteria isolated from fish, seaweed and seawater, but did not specify their optimum growth temperature. It might be assumed that some of these were psychrophilic because several were isolated from codfish which live in relatively cold water. However, the small number of bacteria studied would preclude any conclusions about the status of psychrophilic marine bacteria.

In the study described here all strains except the type strains were isolated from the northwest Atlantic Ocean. Powell (1980) has shown that, in this region, the seasonal temperature change above the thermocline can range between -1°C and 18°C. Morita (1975) has defined psychrophilic bacteria as organisms having an optimal temperature for growth at about 15°C or lower, a maximal temperature for growth at about 20°C, and a minimal temperature for growth at 0°C or below. In the literature a distinction is usually made between psychrophilic bacteria and psychrotrophic bacteria. Psychrophilic bacteria grow well at approximately 0°C whereas psychrotrophic bacteria will grow at low temperatures (approximately 4°C) but may have an optimum temperature for growth of 20°C or higher. It has been shown that populations of bacteria from the northwest Atlantic Ocean near Newfoundland have the growth temperature characteristics of psychrophilic bacteria for most of the year. However, strains isolated from warm surface waters (14°C) in August have been shown to have psychrotrophic properties (F. Mills,

B.Sc. Honours thesis, Memorial University of Newfoundland, St. John's, Newfoundland, in preparation; Hollohan et al., 1982). Therefore, the strains selected for study here are presumed to be either psychrophilic or psychrotrophic. Where only the term psychrophilic is used in this thesis to describe the strains it should be interpreted to mean psychrophilic or psychrotrophic because detailed studies were not done to determine the precise category to which each strain belonged. Since over 90% of the marine environment (by volume) has a temperature below 5°C (ZoBell, 1963), Baumann and Baumann (1981) stated that it would be of considerable interest to know whether the psychrophiles from the ocean differ from previously characterized mesophiles only in their relation to temperature or whether they actually constitute different species. The results of this study would suggest that, although cold ocean bacteria and mesophilic marine bacteria resemble each other in several aspects, there would be justification for the designation of some new taxa. It was also found that clusters conformed largely to the source of the strains, with the strains from seaweed clustering separately from scallop isolates, with several clusters from each source.

MATERIALS AND METHODS

Sources of the strains

The strains used were part of a culture collection of marine bacteria maintained by Dr. J. Gow, Biology Department, Memorial University of Newfoundland.

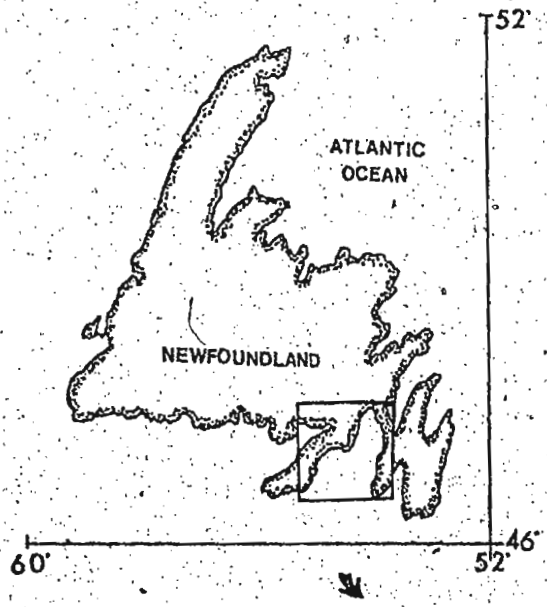
(a) Strains isolated from scallop.

Fifty-six of the strains had been isolated from the homogenized body and viscera of giant scallops (Placopecten magellanicus). These were obtained during a single isolation on May 25, 1977. The site of collection of the scallops was Buffett Harbour, Placentia Bay, Newfoundland (Lat. 47°32'N, Long. 53°28'W) (Fig. 1). The water temperature was 5.8°C and the depth of collection was 16 m. The isolation and a preliminary characterization of these strains has been described by Powell (1978).

(b) Strains isolated from seaweed.

Eighty-four of the strains had been isolated from fronds of Alaria esculenta. In this instance, the strains were from three sub-sets isolated over a period during which natural decomposition was taking place. The site of collection was Logy Bay, Newfoundland (Lat. 47°37'N, Long. 52°40'W) (Fig. 2) at a depth of 10 m. The samples were collected during 1979 on June 13 (water temperature, 5°C), August 15 (water temperature, 6°C) and September 20 (water temperature, 10°C).

Figure 1. Map of Placentia Bay showing the sampling site at Buffett Harbour.


52°
ATLANTIC
OCEAN
NEWFOUNDLAND
60°
46°
52°

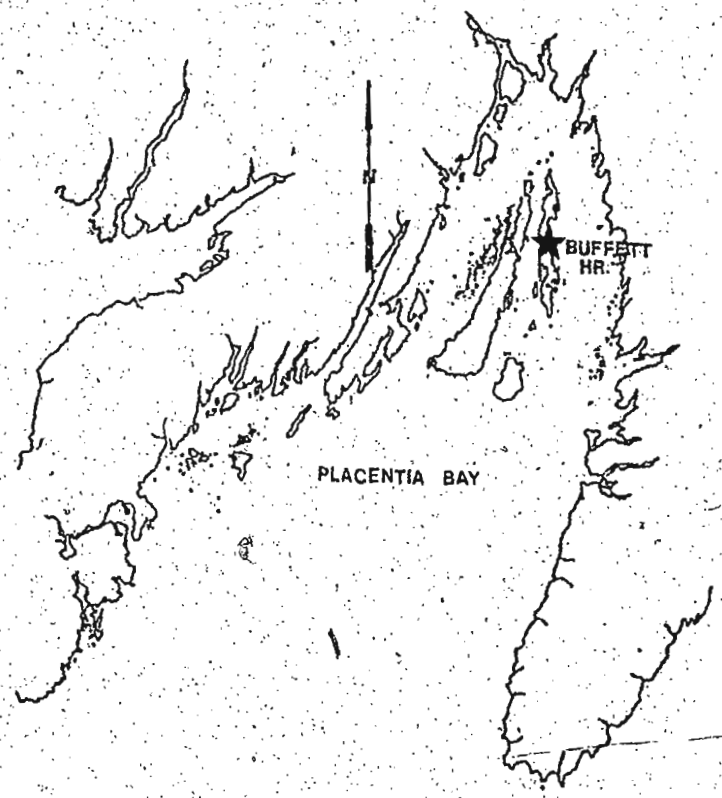
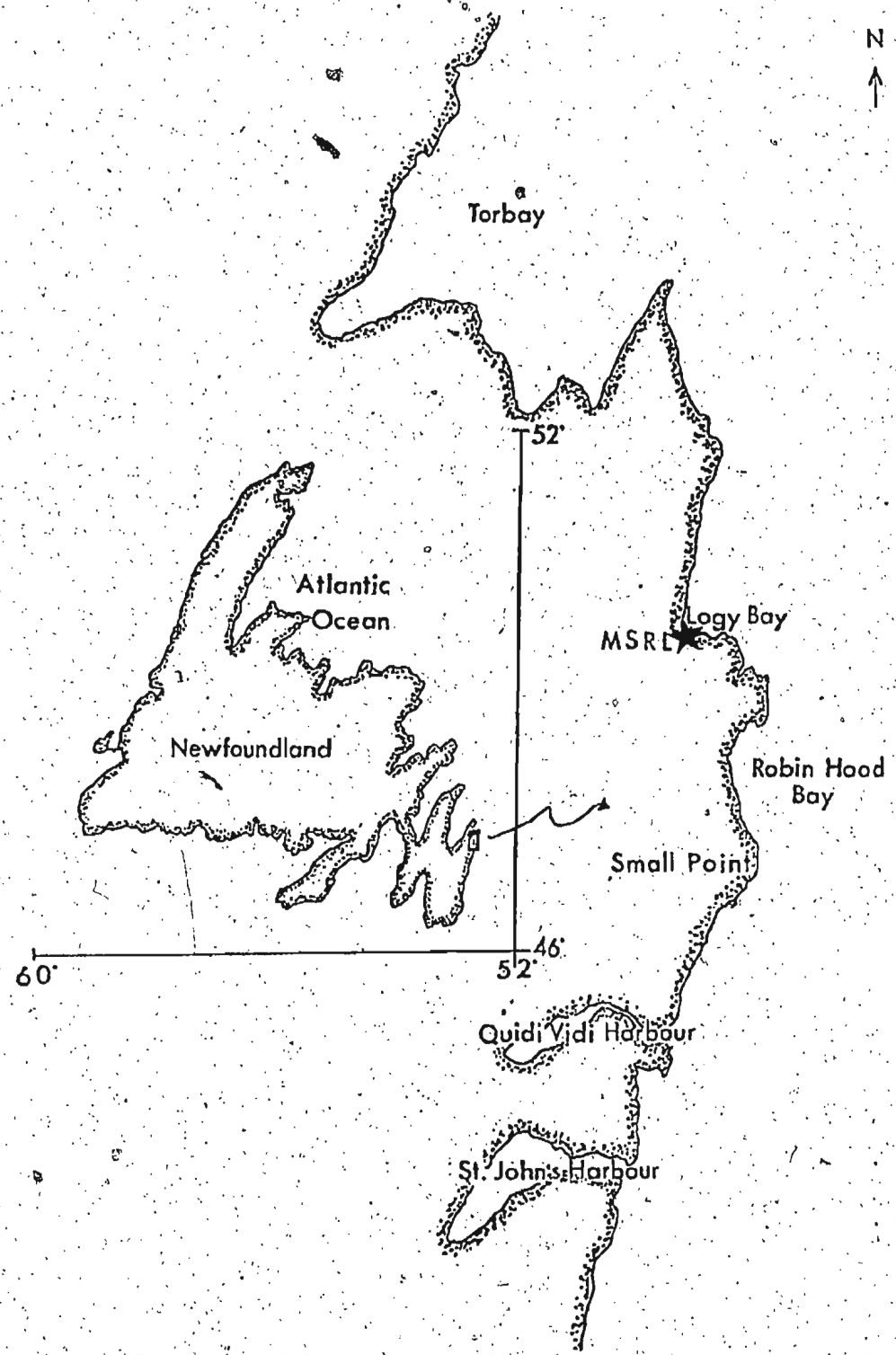

N
BUFFETT
HR.
PLACENTIA BAY

Figure 2. Section of the east coast of the Avalon Peninsula showing the sampling site at Logy Bay.


N
Torbay
52'
Atlantic
Ocean
Logy Bay
MSRL
Newfoundland
Robin Hood
Bay
Small Point
60'
52'46'
Quidi Vidi Harbour
St. John's Harbour

Initially, the blades were collected on June 13 and cut transversely into sections 10 cm long. The portion of the blade used was that located above the meristem and below the tip. Sub-samples were placed in sample chambers made of ABS plastic pipe covered at both ends with plastic mesh. The sample chambers, each containing a weighed sample of A. esculenta blades, were returned to the original collection site at Logy Bay where they were tied to a cable on the sea floor. Twenty-four sample chambers were prepared.

At each sample date blades were taken from two chambers, weighed and homogenized as described by Chan and McManus (1967). Samples were diluted and plated by the spread plate technique (Buck and Cleverdon, 1960). Forty-four bacterial strains were isolated from the June 13 sample, forty-five from the August 15 sample, and thirty from the September 20 sample. These isolates were designated D1-1 to D1-44, D2-1 to D2-45, and D3-1 to D3-30, respectively. Details of the isolation and a preliminary characterization of these strains have been described by Hollohan (1980).

(c) Type cultures.

Sixteen type cultures and/or reference strains were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland. They are listed in Table 1.

Table 1. Type cultures of marine bacteria from the American Type Culture Collection (ATCC), Rockville, Maryland, that were included in this study as control cultures.

Type culture	ATCC #
Alteromonas undina	29660
Alteromonas espejiana	29659
Vibrio vulnificus	27562
Vibrio splendidus	25914
Vibrio nigrapulchrituda	27043
Alteromonas macleodii	27126
Photobacterium angustum	25915
Alcaligenes aquamarinus	14400
Alcaligenes venustus	27125
Pseudomonas nautica	27132
Photobacterium phosphoreum	11040
Alteromonas communis	27118
Alteromonas vaga	27119
Alcaligenes cupidus	27124
Pseudomonas doudoroffii	27123
Pseudomonas marina	25374

Selection of the strains

An objective of this study is to produce a classification of marine, heterotrophic bacteria isolated from the coastal waters of Newfoundland. In order to reduce the scope of the study only strains that required Na^+ for growth were selected. These are true marine bacteria as defined by MacLeod (1965, 1968).

Of the 96 bacterial strains isolated by Powell (1978) from P. magellanicus 80 required Na^+ for growth. This was 83% of the isolates. Fifty-six of the 80 Na^+ - requiring strains (70%) were selected for further characterization. From scallops, marine fermentative bacteria made up the minority (42.5%) of the Na^+ - requiring strains. All but one of these strains were included in the current study. Oxidative strains made up the remaining Na^+ - requiring bacteria. Slightly more than half (24) of the oxidative strains were included in the current study. Powell (1978), using a limited number of tests, had found that the oxidative strains comprised two major bacterial groups. The oxidative strains selected for inclusion in the present study were selected from the two groups.

Of the 119 strains isolated from A. esculenta 90% required Na^+ for growth. Eighty-four (71%) were selected for further characterization. Therefore, the proportion of Na^+ - requiring bacteria from scallop and seaweed included in the current study was approximately the same. However,

from seaweed only 19 (16%) of the Na^+ - requiring strains were oxidative. All of these were included in the present study. Sixty-four (60%) fermentative Na^+ requiring strains from A. esculenta were selected for further study. These were selected in proportion to their abundance in each of the three sub-sets isolated during the course of study of the degradation of the fronds.

The ATCC type strains included in the study were selected so that each currently accepted genus of marine bacteria would be represented. Some were reference strains included to provide the positive and negative controls for the individual characterization tests.

In summary, 156 strains of Na^+ - requiring marine bacteria were included in this study. Fifty-six of these had been isolated from the scallop Placopecten magellanicus, 84 from the seaweed Alaria esculenta, and 16 were type cultures.

A list of the strains used in this study is given in Appendix A. The number assigned to each strain in this study is given along with the corresponding number from the appropriate study of either Powell (1978) or Hollohan (1980). This Appendix will facilitate the cross-reference of data in this thesis with that of the preliminary studies.

Media

Complex media were prepared from dehydrated products and ingredients purchased from either Difco (Detroit, Michigan), Baltimore Biological Laboratory (BBL).

(Baltimore, Maryland), or Oxoid (Great Britain). Because marine bacteria have specific ion requirements the culture media were rehydrated with either 75% natural aged seawater or a salts solution of the following composition per litre: 17.5 g NaCl, 12.05 g $MgSO_4 \cdot 7 H_2O$, 0.75 g KCl, 0.147 g $CaCl_2 \cdot 2H_2O$, 6.05 g tris (hydroxymethyl) aminomethane (Tris), 0.132 g $(NH_4)_2 HPO_4$ and 10 mg $Fe (NH_4)_2 (SO_4)_2$. British Drug Houses (Canada) Limited (BDH) Analar and Fisher Scientific Company (New Jersey) certified A.C.S. reagents were used to make the salts solution which was prepared in two parts. The NaCl, $MgSO_4 \cdot 7 H_2O$, KCl and $CaCl_2$ were dissolved in distilled water, at double strength, separately from the remaining ingredients. This solution was called artificial seawater (ASW). The remaining ingredients were dissolved, at double strength, in the following order. Tris was dissolved and adjusted to pH 7.5 with conc. HCl. The $(NH_4)_2 HPO_4$ was added and the solution was brought up to full volume less 10 ml. The $Fe (NH_4)_2 (SO_4)_2$ was dissolved in 10 ml distilled H_2O and added last. This solution was called buffered salt solution (BSS). The salts solution, called buffered artificial seawater (BASW), was made by adding equal quantities of ASW and BSS. When the ingredients were mixed as described, a salts solution was obtained that was precipitate-free.

Solidified media were prepared by adding either Oxoid Technical Agar No. 3 at 1.2% (w/v) or Oxoid Purified Agar at 1.0% (w/v). Complex media were prepared with

technical grade agar. Defined media prepared with purified agar are noted.

Cultures were maintained on a medium described by Colwell and Wiebe (1970). It had the following composition per litre: 1.0 g Proteose Peptone No. 3 (Difco), 1.0 g Yeast Extract (Difco) and 12.0 g technical agar dissolved in either 75% natural aged seawater or BASW. This medium is referred to as Colwell's seawater medium or Colwell's BASW medium, respectively.

To test for Na^+ requirement the cultures were compared for growth on two defined media that were similar except that NaCl was replaced by an equimolar concentration of KCl in one of them. The organic substrates were 0.1% glucose and 0.1% L-glutamate. These media were solidified with purified agar.

Yeast extract broth (YEB) was made by adding 0.5% (w/v) Yeast Extract (Difco) to BASW. This medium is similar to one described by Baumann and Baumann (1981).

Luminescence medium (LM) consisted of BASW containing 0.3% (v/v) glycerol, 5.0 g/ℓ yeast extract, 5.0 g/ℓ tryptone, 1.0 g/ℓ $CaCO_3$ and 12.0 g/ℓ technical agar (Baumann and Baumann, 1981).

The media used to test for growth on single carbon sources were prepared by adding most of the carbon compounds at a concentration of 0.1% (w/v) to BASW. The exceptions were the sugars, which were added at a concentration of 0.2% (w/v). The media were solidified with purified agar. A

list of the organic compounds tested as sole sources of carbon and energy is given in Table 2. The method of sterilizing each carbon compound is given in the table. Some substrates were autoclaved in the BASW plus agar, but for the majority a concentrated solution of the substrate was filter - sterilized (Millipore, 0.45 μm pore size) and then added to BASW plus agar that had been sterilized by autoclaving and then cooled to 45°C. Further details about the preparation and sterilization of organic compounds can be found in an article by Palleroni and Doudoroff (1972).

Modifications of the media listed above were required to perform some of the individual characterization tests that are described next. These modifications are given with the appropriate test procedures.

Characterization tests

A number of characterization tests were performed. The majority of the tests have been described in detail in one or more of three general references: Colwell and Wiebe (1970), Baumann and Baumann (1981), and Smibert and Krieg (1981). The appropriate reference(s) is cited for each method used. Exceptions to the methods and/or further explanations are given. When incubation of tests or cultures was required it was done at 20°C unless otherwise stated.

(a) Gram reaction and morphology: Smears of 18-24 h cultures were stained and examined, using light

Table 2: List of organic compounds tested as sole sources of carbon and energy. The method of sterilization is given in parentheses.

(1) Carbohydrates and sugar derivatives (membrane filtration):

D-ribose	melibiose	saccharate
D-arabinose	lactose	inulin
L-arabinose	D-fucose	mucate
D-xylose	L-rhamnose	D-galacturonate
sucrose	D-glucose	D-gluconate
trehalose	D-mannose	D-glucuronate
maltose	D-galactose	salicin
cellobiose	D-fructose	

(2) Fatty acids (autoclaving):

acetate	isobutyrate	heptanoate
propionate	isovalerate	glycocholate
butyrate	pelargonate	valerate

(3) Dicarboxylic acids (autoclaving):

oxalate	succinate	adipate
malonate	maleate	pimelate
formate	fumarate	azelate
suberate	sebacate	

Table 2 continued ...

(4) Hydroxyacids (autoclaving):

DL-malate	D-(-)-tartrate
L-malate	D-(+)-tartrate
DL-glycerate	meso-tartrate
DL-lactate	glycolate
DL-β-hydroxybutyrate	

(5) Misc. organic acids (membrane filtration):

citrate	aconitate
α-ketoglutarate	itaconate
pyruvate	

(6) Alcohols (no sterilization required):

ethanol	n-butanol
isopropanol	propanol

(7) Polyalcohols and glycols (membrane filtration):

erythritol	sorbitol
mannitol	inositol
adonitol	glycerol*

*sterilized by autoclaving

Table 2 continued ...

(8) Non-nitrogenous aromatic and other cyclic compounds (membrane filtration):

D-mandelate	phthalate
L-mandelate	phenylacetate
benzoate	quinate
m-hydroxybenzoate	p-hydroxybenzoate

(9) Aliphatic amino acids (autoclaving):

glycine	L-aspartate
L-α-alanine	L-lysine
D-α-alanine	DL-arginine
β-alanine	DL-α-aminobutyrate
L-serine	DL-α-aminovalerate
L-threonine	γ-aminobutyrate
L-leucine	δ-aminovalerate
L-isoleucine	N-acetylglucosamine
DL-norleucine	L-glutamate
L-valine	DL-citrulline
DL-ornithine	

Table 2 continued ...

(10) Amino acids and related compounds containing a ring structure (membrane filtration):

L-histidine	L-phenylalanine
L-proline	L-tryptophan
L-tyrosine*	D-tryptophan
DL-kynurenine	kynurenate
p-aminobenzoate	

*sterilized by autoclaving

(11) Amines (sintered glass filtration):

ethanolamine
benzylamine
putrescine

(12) Misc. nitrogenous compounds (membrane filtration):

betaine	creatine	nicotinamide
sarcosine	hippurate	allantoin
acetamide	nicotinate	adenine

microscopy, for gram reaction and morphology (Colwell and Wiebe, 1970).

(b) Motility: This was determined by examining the cultures as wet mounts using phase contrast optics (Colwell and Wiebe, 1970).

(c) Flagella stain: The cultures were grown in Colwell's BASW medium for 18 - 24 h. The flagella were stained by the method of Mayfield and Inniss (1977) and observed with phase contrast optics.

(d) Leifson MOF (oxidation-fermentation) test: Bacto-MOF medium (Difco) was supplemented with 1.0% (w/v) filter-sterilized glucose. Tubes were stab-inoculated in duplicate and one of the tubes was overlaid with sterile mineral oil. After 6 days incubation the reactions were read as follows. Oxidative organisms produced acid in the aerobic tube only. Fermentative organisms produced acid in both the aerobic and anaerobic tubes (Leifson, 1963).

(e) Na^+ requirement: The strains were tested for growth on the medium in which NaCl was replaced by KCl. The medium was inoculated with washed cell suspensions of the test organisms. NaCl was omitted from the BASW used to wash and suspend the cells. A control medium containing NaCl was also inoculated. The plates were observed for growth after 2 weeks incubation.

(f) Oxidase test: A sheet of Whatman's No. 1 filter paper was placed in a petri dish and several drops of a 1% aqueous solution of tetramethyl-paraphenylene-diaminedihydrochloride in 0.2% ascorbic acid were added to the paper. The test colony was removed and streaked onto the reagent - impregnated paper with a platinum loop. Oxidase-positive organisms turned the filter paper purple within 60 seconds. The test was negative if there was no reaction within 60 seconds (Skerman, 1967).

(g) Catalase test: Several drops of 3% H_2O_2 were added to a 48 h slant culture (Colwell's BASW medium) of each test organism. The evolution of bubbles within 5 minutes was a positive test (Skerman, 1967).

(h) Arginine dihydrolase: The cultures were stab-inoculated into BASW lacking Tris-HCl (ph 7.5) and containing 10 g/ℓ L-arginine, 1 g/ℓ Proteose Peptone No. 3, 10 mg/ℓ phenol red and 2 g/ℓ technical agar (Baumann and Baumann, 1981). About 2 ml of 2% agar, which was autoclaved and cooled to 40°C, was carefully layered over the medium to form an agar plug. Another tube, identical except for the omission of the L-arginine, was used as a control. The cultures were incubated for 4 days and observed periodically for a difference in colour due to alkali production in the L-arginine - containing medium. In this way, all potential arginine dihydrolase organisms were easily detected. However,

the above test has the disadvantage of being a nonspecific test for the arginine dihydrolase system since an increase in pH can also be due to a decarboxylation or deamination of L-arginine. Therefore, all positive organisms were checked for the presence of a constitutive arginine dihydrolase system by the procedure of Stanier et al. (1966). The organisms were grown in 500 ml of BASW containing 0.2% succinate. The pH of the medium was adjusted to 7.5 with 1N NaOH. The cells were harvested by centrifugation and suspended in ½ ASW containing 50 mM Tris-HCl (pH 7.5) to an OD of 200 Klett units (green filter). The suspensions were purged by bubbling nitrogen gas through them for several minutes. One millilitre of 0.001 M L-arginine monohydrochloride was added. After purging with N_2 again, the tubes were stoppered and incubated at 30°C for 2 h, and then heated at 100°C for 15 minutes. After removing the cells by centrifugation, the arginine in the supernatant was determined as follows. One millilitre of each sample was mixed with 2 ml of developing solution, 1 ml 3 N NaOH, and 6 ml distilled water. The tubes were read after 30 minutes against a blank prepared without arginine, using a colorimeter equipped with a green filter (540 nm); the readings were compared to those obtained with an uninoculated control containing arginine. A positive test was shown by the disappearance of some or all of the arginine. The developing

solution was prepared by mixing 20 ml of 25% (w/v) α-naphthol in n-propanol with 2.5 ml of stock diacetyl solution, and diluting the mixture to 100 ml with n-propanol.

(i) Denitrification: The ability to denitrify was tested in YEB containing 100 mM Tris-HCl (pH 7.5), 0.1% succinate, 0.1% acetate, 0.1% lactate, 0.2% technical agar, and 0.3% $NaNO_3$. After a 48 h incubation in 5 ml of the medium, the cultures were stab-inoculated into 10 ml of the same medium and overlaid with an agar plug. The cultures were observed for 6 days and a positive test was indicated by turbidity and gas production (Baumann and Baumann, 1981).

(j) Nitrate to nitrite: The conversion of NO_3 to NO_2 in denitrification medium was tested by the starch-iodine spot test for nitrite (Skerman, 1967). A starch-iodine solution was prepared by dissolving 2.0 g $ZnCl_2$ in 10 ml H_2O, boiling, and adding 4.0 g starch. The solution was then diluted to 100 ml with distilled water. After one week the solution was filtered and an equal volume of a 0.2% aqueous solution of KI was added to the filtrate. A hydrochloric acid solution was prepared by adding 16 ml conc. HCl to 84 ml distilled water. Using clean Pasteur pipettes, one drop of each reagent was placed on a clean glass slide and one drop of culture was added. A blue colour indicated the presence of nitrite.

(k) Voges-Proskauer test: The strains were grown in test tubes containing 5 ml YEB with 1% (w/v) D-glucose. After a 72 h incubation the Voges-Proskauer test was performed (Blazevic and Ederer, 1975). One millilitre of culture was removed to a clean test tube and then 0.6 ml of 5% (w/v) α-naphthol in absolute ethanol was added and mixed well. Then 0.2 ml of 40% aqueous KOH was added and the tubes were shaken. A positive test was shown by the appearance of a red colour within 5 minutes.

(l) Growth temperatures: Washed cell suspensions of bacteria were inoculated into YEB in test tubes and incubated in circulating water baths set at 4°C, 35°C, and 40°C (Baumann and Baumann, 1981). The tubes were observed for growth periodically for 3 weeks, 5 days, and 3 days, respectively. Visible turbidity was considered a positive test (Colwell and Wiebe, 1970).

(m) Luminescence: The strains were streaked onto plates of LM and incubated at 15°C for up to 36 h. The plates were checked for luminescence every 3 h at 12-36 h by observing them in a darkened room. Fifteen minutes were allowed for the eyes to adjust to the dark (Baumann and Baumann, 1981).

(n) Extracellular enzymes: The production of an extracellular chitinase, laminaranase, alginase, amylase, gelatinase, cellulase and lipase was determined by

inoculating the appropriate medium with a patch of cells and observing for a zone of hydrolysis beyond the limits of growth. Several strains could be tested on the same plate.

Chitinase production was tested on Colwell's BASW medium overlaid with 10-15 ml of modified Colwell's BASW medium containing about 5 g/ℓ colloidal chitin and 2.5 g/ℓ yeast extract. The chitin was prepared as described by Berger and Reynolds (1958).

Laminaranase activity was tested on Colwell's BASW medium overlaid with medium containing 4% (w/v) laminaran.

Alginase activity was tested on Colwell's BASW medium overlaid with Colwell's medium containing 20 g/ℓ sodium alginate.

An overlay method was not needed to detect the presence of an extracellular amylase, gelatinase, and lipase. To test for these exoenzymes, Colwell's BASW medium was supplemented with 2 g/ℓ starch, 20 g/ℓ gelatin, and 10 ml/ℓ polyethylene sorbitan mono-oleate (Tween-80), respectively.

The hydrolysis of chitin, alginate, or laminaran resulted in a zone of clearing, whereas lipase activity on Tween-80 plates was detected by the appearance of a precipitate of calcium oleate. All plates were observed for a period of 6 days.

After incubation for 48 h, the starch plates were flooded with Lugol's iodine solution. If starch was hydrolyzed, only the colour of the iodine was seen on the plates. If starch was not hydrolyzed it combined with the iodine to give a blue-purple colour.

After an incubation of 48 h, the gelatin plates were flooded with 30% trichloroacetic acid as a protein precipitant. Positive tests showed a clear zone around the area of growth and a border of white precipitate around the clear zone.

The production of cellulase (glucanase) was tested on Colwell's BASW medium containing 1% carboxymethyl-cellulose (Teather and Wood, 1981). After 48 h incubation, the plates were flooded with a 1 mg/ml solution of Congo red in distilled water. After 15 minutes the Congo red was discarded and the plates were flooded with a 1 M solution of NaCl for a further 15 minutes. A clear zone around the area of growth indicated a positive test.

(o) Agar digestion: This was tested by growing the strains on modified Colwell's BASW containing 1% (w/v) purified agar instead of 1.2% (w/v) technical agar. A depression around the area of growth indicated a positive test.

(p) Poly-β-hydroxybutyrate (PHB) accumulation: The strains were grown in BASW containing 0.02% (w/v) $(NH_4)_2SO_4$ and 0.4% (w/v) DL-β-hydroxybutyrate for 48 h and then tested

for the presence of PHB (Williamson and Wilkinson, 1958; Slepecky and Law, 1960). Two millilitres of culture were put in a conical glass centrifuge tube and 8 ml commercial 5% hypochlorite (Clorox bleach) added. After 24 h the mixture was centrifuged to collect the PHB granules. The sediment was washed by suspending it in 10 ml distilled water, centrifuging, and discarding the supernatant fluid. The sediment was washed once again with 10 ml distilled water, twice with 10 ml portions of acetone, and finally twice with 10 ml portions of diethyl ether. The sediment was dried and 2 ml of conc. H_2SO_4 added. The tubes were placed in a boiling water bath for 10 minutes and then cooled to room temperature. The absorption spectrum between 255 and 215 nm was determined using a UV-210 double-beam scanning spectrophotometer (Bausch and Lomb). Samples were read against a blank of plain conc. H_2SO_4. A positive test was the occurrence of an absorption peak at 235 nm, due to crotonic acid which is formed by the action of sulfuric acid on PHB.

(q) Aromatic ring cleavage: The strains were grown in BASW containing 0.1% (w/v) p-hydroxybenzoate, 0.1% (w/v) quinate, or 0.15% (w/v) sodium benzoate, and tested for the mechanism of aromatic ring cleavage (Kilby, 1948; Stanier et al., 1966). The cells were harvested by centrifugation and a very heavy suspension was made in 2 ml of 0.2 M Tris buffer (2-amino-2-hydroxymethyl-1,3-

propanediol), pH 8.0. The tubes were shaken with 0.5 ml toluene and then 3.5 mg protocatechuate were added. A yellow colour within a few minutes indicated meta cleavage. If no colour appeared, the tubes were shaken at 30°C for 1 h followed by the addition of 1.0 g $(NH_4)_2SO_4$, 1 drop of 1.0% (w/v) sodium nitroferricyanide, and 0.5 ml ammonia solution (specific gravity 0.880 or 28-30%). A purple colour indicated ortho cleavage.

(r) Nutritional screening: The strains were screened for their ability to utilize 117 carbon compounds as sole sources of carbon and energy. The list of carbon compounds tested has been given in Table 2. The strains were grown overnight on plates of Colwell's BASW medium and cell suspensions, washed in BASW, were inoculated onto the test media using a 25-point inoculating template (Lovelace and Colwell, 1968; Sneath and Stevens, 1967). The plates were scored after 48 and 96 h of incubation. Unmistakable visible growth on the plates was considered positive. Each test series included a control medium of BASW which lacked a carbon and energy source. This showed any growth due to carry-over of nutrients from the original culture medium. The strains were also grown on plates of Colwell's BASW medium to ensure that the cultures were viable.

Electron microscopy

The following representative strains were examined by electron microscopy to confirm the type of flagellation: 21, 44, 59, 70, 93 and 122 (Appendix A, Table 1). The strains were prepared by using a modification of the technique described by Baumann and Baumann (1981).

Colwell's seawater broth (10 ml) was inoculated from a slant and incubated overnight on a shaker. About 0.5 ml of this culture was transferred into 10 ml Colwell's seawater broth and when light turbidity was detected (approximately 8 h incubation) the culture was poured into a centrifuge tube containing 0.1 ml neutralized (pH 7.5) 37% formaldehyde (formalin). After a light centrifugation (about 10 min. at 3,000 x g), the supernatant was decanted and 10 ml distilled water was added slowly to the pellet. This allowed the cells to suspend without agitation. The cells were washed and suspended two additional times. After the final wash the cells were suspended in 1 ml distilled water. This suspension was negatively stained and observed by electron microscopy.

The stain used was a saturated aqueous solution of uranyl acetate. The stain and bacterial suspension was mixed 1:1 in a conical test tube. A small drop of this mixture was then placed onto a 200 mesh size carbon - Formvar coated copper grid using a clean pasteur pipette. After approximately a minute the grid was touched against a clean piece of filter paper (Whatman No. 2) to drain away

excess liquid. This left a thin film of solution on the grid which dried rapidly. Each grid was observed using a Zeiss EM 9 A electron microscope. A more detailed description of negative staining techniques may be found in Haschemeyer and Meyers (1972).

Determination of moles % G + C of DNA

The procedures used to isolate DNA from representative strains and to determine moles % G + C have been described by Johnson (1981). Only the steps that may be expected to differ between laboratories will be described here. The strains were grown in 10 ml Colwell's seawater medium in 50 ml erlenmeyer flasks. After approximately 8 h incubation at 20°C on a shaker, the 10 ml cultures were used to inoculate 150 ml fresh medium in 250 ml erlenmeyer flasks. These flasks were incubated overnight (about 18 hr) with shaking and then the cells were harvested by centrifugation.

The cells were suspended in 25 ml saline - EDTA buffer. The hydroxylapatite (HTP) method was used to isolate the DNA. All steps described by Johnson (1981) were carried out. As suggested the cells were treated with pronase in addition to RNase and 20% SDS. One extraction with water-saturated phenol was sufficient to denature and separate the protein fraction. It was not necessary to extract DNA a second time from the lysate in order to obtain a satisfactory yield of purified DNA.

Moles % G + C was determined by the thermal melting point (Tm) method as described by Johnson (1981). E. coli DNA (A grade) from Calbiochem-Behring Corp. was used as the standard and was included in each set of determinations. Melting point was determined using the Tm analysis system of Beckman Instruments Division, Ca. The components of this system are used in conjunction with the DU-8 UV-Visible Computing Spectrophotometer (Beckman Instruments). A Compuset computer module is programmed to control a six-positioned temperature-controlled sample holder and to perform data reduction for the accessory. Using this system it was possible to include a blank, the reference DNA, and four unknowns in each sample run. Moles % G + C was calculated from the Tm values generated by the Compuset computer module.

Numerical taxonomy

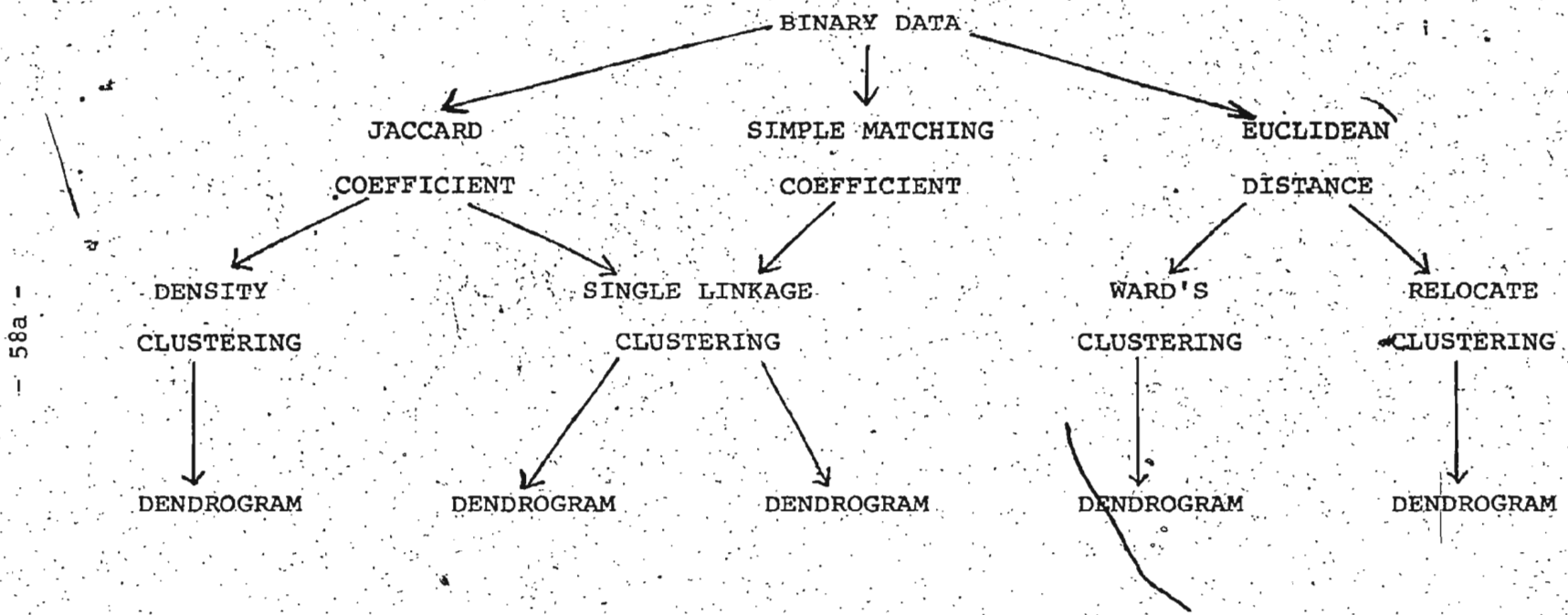
A flow chart outlining the computer programs used in this study is given in Figure 3. The data matrix consisted of 149 OTU (140 isolates and 9 type strains) and 112 tests. Tests which yielded results that were either all positive or all negative for the strains were not used. Those that yielded both positive and negative scores were converted to a numerical format as follows: a positive result (+) was recorded as 1 and a negative result (-) was recorded as 0.

The cluster analysis programs used are contained in the "Clustan" package of programs (Wishart, 1978). They are:

1. The Jaccard similarity coefficient with single linkage clustering.
2. The simple matching coefficient (SMC) with single linkage clustering.
3. The Jaccard similarity coefficient with density clustering.
4. Euclidean distance coefficient with Ward's clustering.
5. Euclidean distance coefficient with relocate clustering.

Each of the five cluster analysis programs yielded a dendrogram (tree diagram) in which distinct clusters of strains could be identified.

Figure 3. Flow chart outlining the cluster analysis programs used to produce the OTU classification.


BINARY DATA
JACCARD COEFFICIENT
SIMPLE MATCHING COEFFICIENT
EUCLIDEAN DISTANCE
DENSITY CLUSTERING
SINGLE LINKAGE CLUSTERING
WARD'S CLUSTERING
RELOCATE CLUSTERING
DENDROGRAM
DENDROGRAM
DENDROGRAM
DENDROGRAM
DENDROGRAM

All computations were carried out using the IBM 370 computer available through Memorial University Computing Center.

RESULTS

Preliminary characterization of the strains (OTU)

The one hundred and forty-nine bacterial strains (OTU) were tested to ensure that they were gram-negative, motile, rod-shaped bacteria that required Na^+ for growth. One hundred and one of the OTU (68%) fermented glucose. The remaining 48 OTU used glucose oxidatively.

All of the OTU were screened for their ability to use 117 organic compounds as sole sources of carbon and energy. Only L-glutamate was used by every OTU. The 25 compounds not used by any OTU are listed in Table 3. Ninety-one compounds were used by one or more OTU but not by all of them. A list of these substrates and the results obtained for each OTU is given in Appendix A, Tables 2 and 3.

All OTU had polar monotrichous flagella when grown in broth and all were Voges-Proskauer negative. The OTU that were capable of aromatic ring cleavage did so by the ortho pathway. Only one OTU showed luminescence and this was a type culture. Similarly, hippurate was utilized by only a type culture.

The data matrix used in the numerical analysis is given in Appendix A, Table 3. The matrix includes all characters which gave both positive and negative results. These are listed in Appendix A, Table 2. Characters which

Table 3. Sole sources of carbon and energy not used by any OTU.

D-fucose	m-hydrozybenzoate
inulin	creatine
L-tartrate	adenine
formate	acetamine
oxalate	nicotinamide
pimelate	D-mandelate
D-tryptophan	L-mandelate
DL-kynurenine	erythritol
kynurenate	meso-tartrate
p-aminobenzoate	D-tartrate
ethanolamine	phthalate
benzylamine	glycocholate
isopropanol	

were either all positive or all negative were excluded from the numerical analysis.

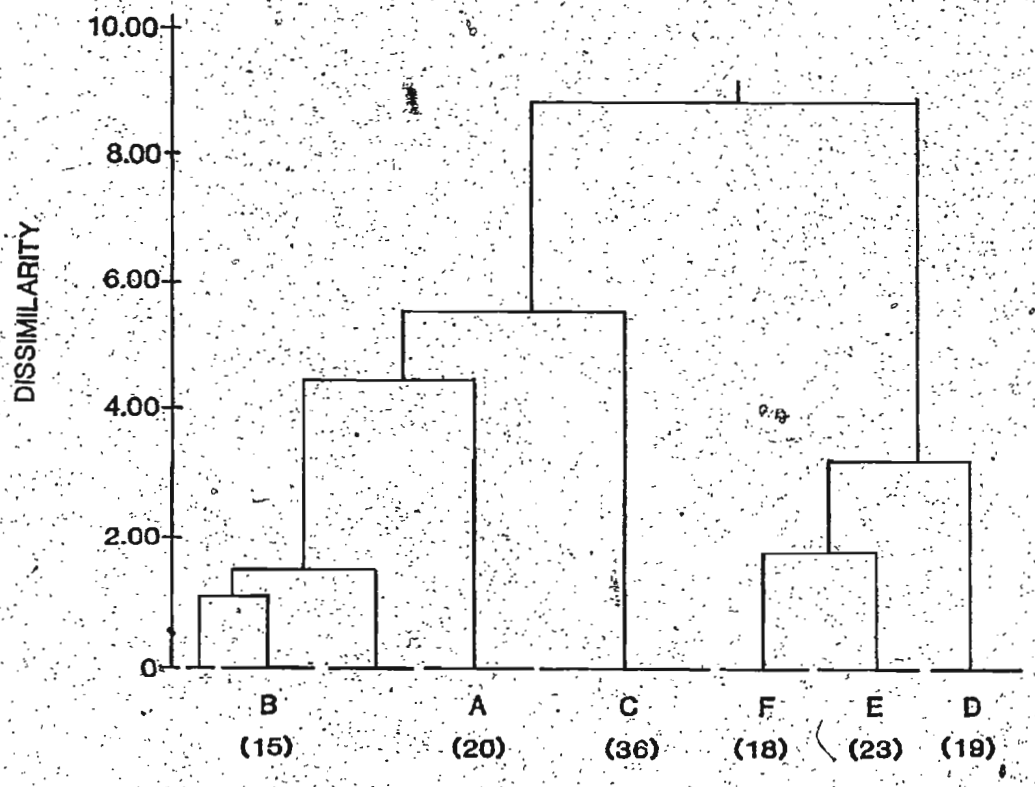
Determination of robustness of classification

Five cluster analysis programs were used. These have been outlined in Materials and Methods. The OTU classifications which resulted from the five cluster analysis programs are given in dendrograms (Figures 4 to 8). These dendrograms have been simplified by representing clusters of OTU as large closed triangles. The base of the triangle is in proportion to the number of OTU in the cluster. The complete dendrograms showing the clustering of each OTU are given in Appendix A.

Dendrograms are strictly hierarchical due to the clustering methods used. The vertical scale gives the similarity or dissimilarity measures at which OTU or clusters of OTU merge. The horizontal scale has no quantitative meaning because OTU are arranged to represent the clusters manifested by the similarity or distance measures. A dendrogram should be regarded as a mobile with the horizontal axis free to rotate and it should be interpreted as representing phenetic similarity and not phylogenetic relationships (Dabinett, 1976).

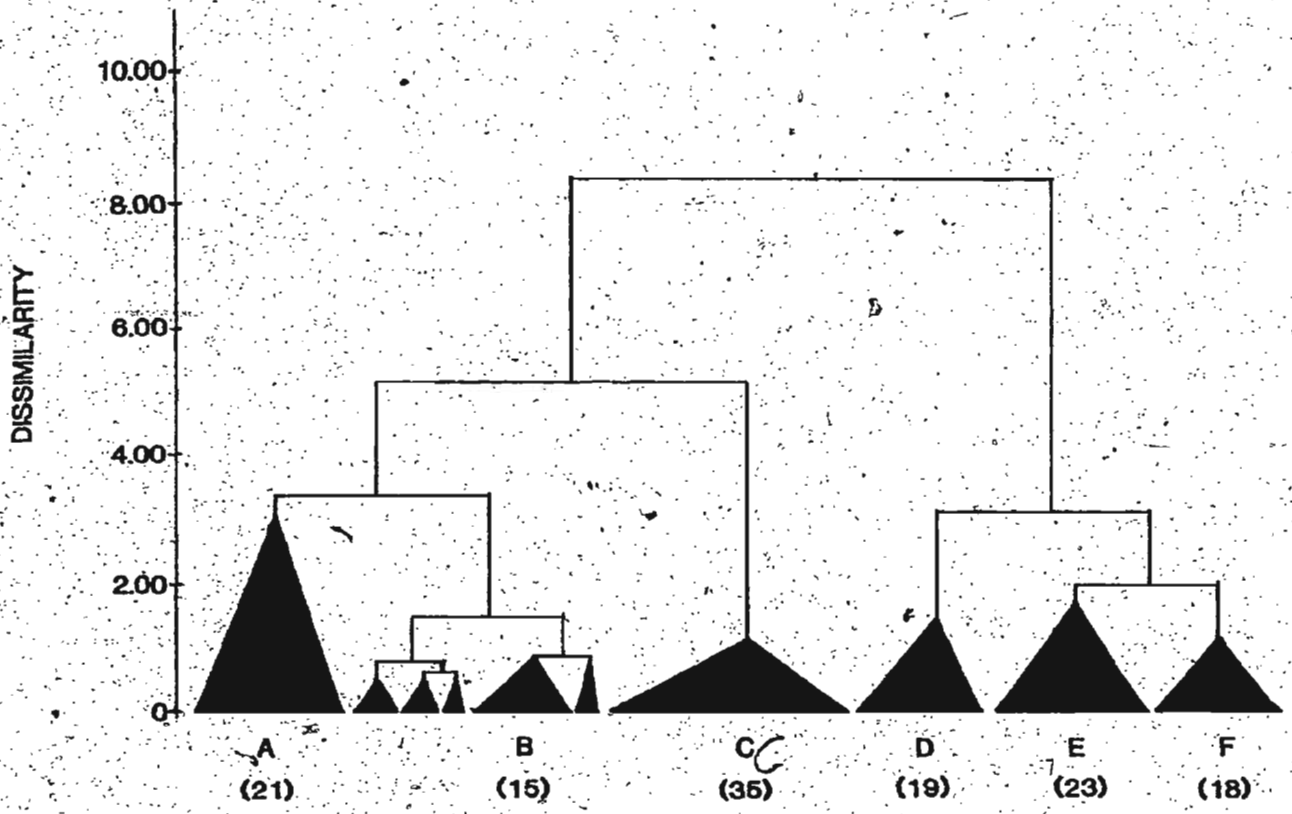
The dendrograms depicted in Figures 4 to 8 were compared with respect to the rank order of the arrangements of the clusters to see whether any consistent hierarchical clustering patterns emerge. Figures 9 to 13 represent

Figure 4. Simplified version of the dendrogram produced by the Euclidean distance coefficient with relocate clustering.



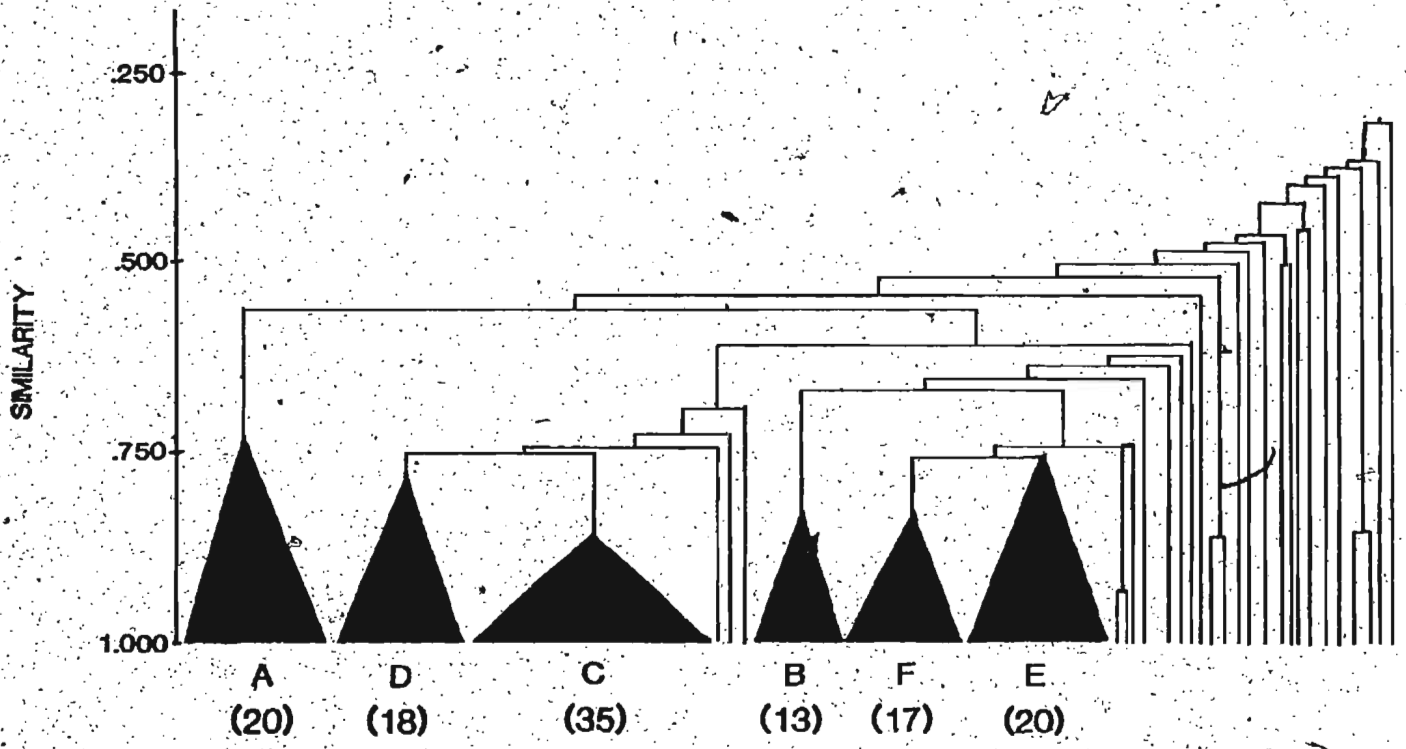
EUCLIDEAN DISTANCE , RELOCATE CLUSTERING

Figure 5. Simplified version of the dendrogram produced by the Euclidean distance coefficient with Ward's clustering.



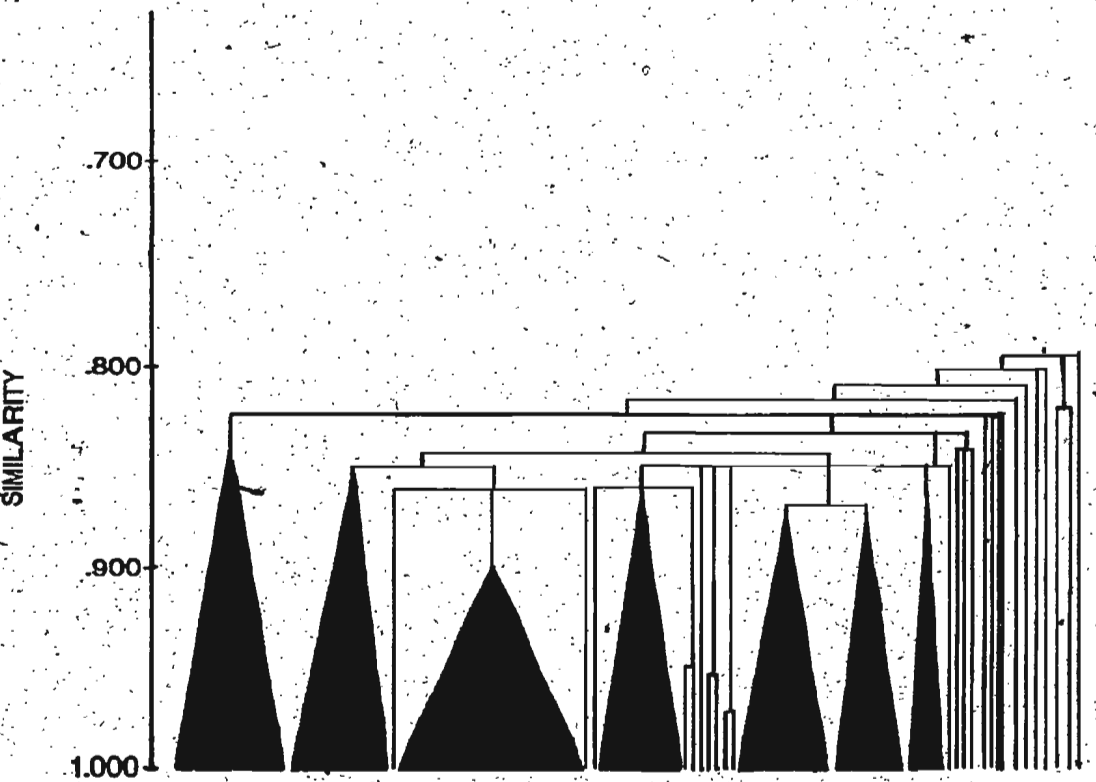
EUCLIDEAN DISTANCE , WARD'S CLUSTERING

Figure 6. Simplified version of the dendrogram produced by the Jaccard coefficient with single linkage clustering.



JACCARD COEFFICIENT, SINGLE LINKAGE CLUSTERING

Figure 7. Simplified version of the dendrogram produced by the simple matching coefficient with single linkage clustering.

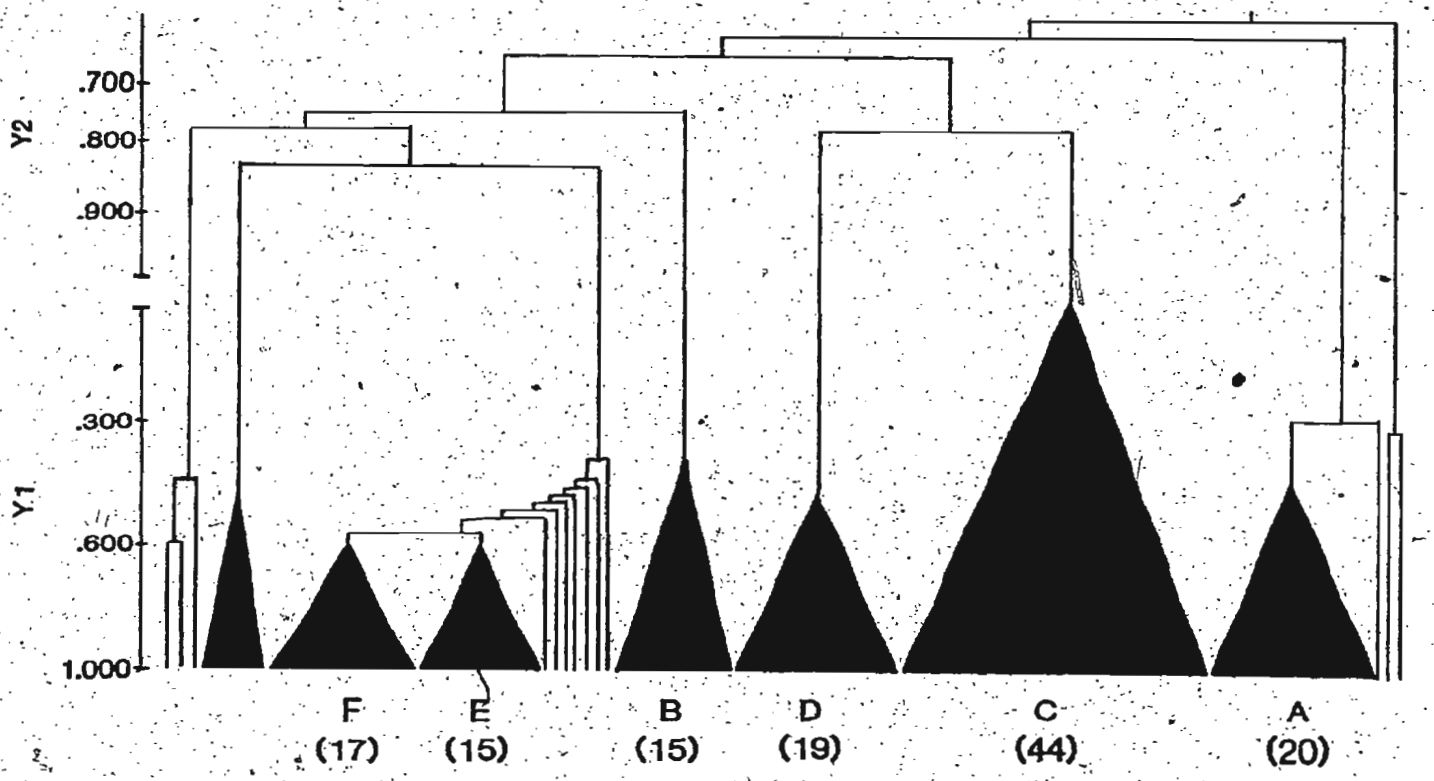


SIMPLE MATCHING COEFFICIENT, SINGLE LINKAGE CLUSTERING

Figure 8. Simplified version of the dendrogram produced by the Jaccard coefficient with density clustering.

Y1 = similarity values, density clustering.

Y2 = similarity values, single linkage clustering.



JACCARD COEFFICIENT, DENSITY CLUSTERING

further simplifications of the five dendrograms, showing the hierarchical arrangement and similarity relationships of the clusters.

It is apparent from Figures 9 and 10 that an identical hierarchical arrangement of the clusters is obtained when both relocate and Ward's clustering methods are used. In both cases the Euclidean distance coefficient was used. Essentially, these two methods divide the 140 seaweed and scallop OTU into two broad groups. One of these groups contains clusters A, B and C. Both clustering methods fuse clusters A and B first, followed by the fusion of the large A-B cluster with cluster C. The other broad group of OTU distinguished by relocate and Ward's clustering methods consists of clusters D, E and F. Both clustering methods first fuse cluster E (two groups) with cluster F, and the resulting E-F cluster then fuses with cluster D (two groups). This broad group, consisting of clusters D, E and F, then fuses with the other broad group, consisting of clusters A, B and C, at a very great distance measure.

The hierarchical arrangements of the clusters obtained when the Jaccard and simple matching coefficients were used, both with single linkage clustering, and the Jaccard coefficient with density clustering, are given in Figures 11 to 13, respectively. The hierarchical patterns obtained from the Jaccard coefficient with density clustering and the simple matching coefficient with single

Figure 9. Hierarchy of clustering produced by the Euclidean distance coefficient with relocate clustering.

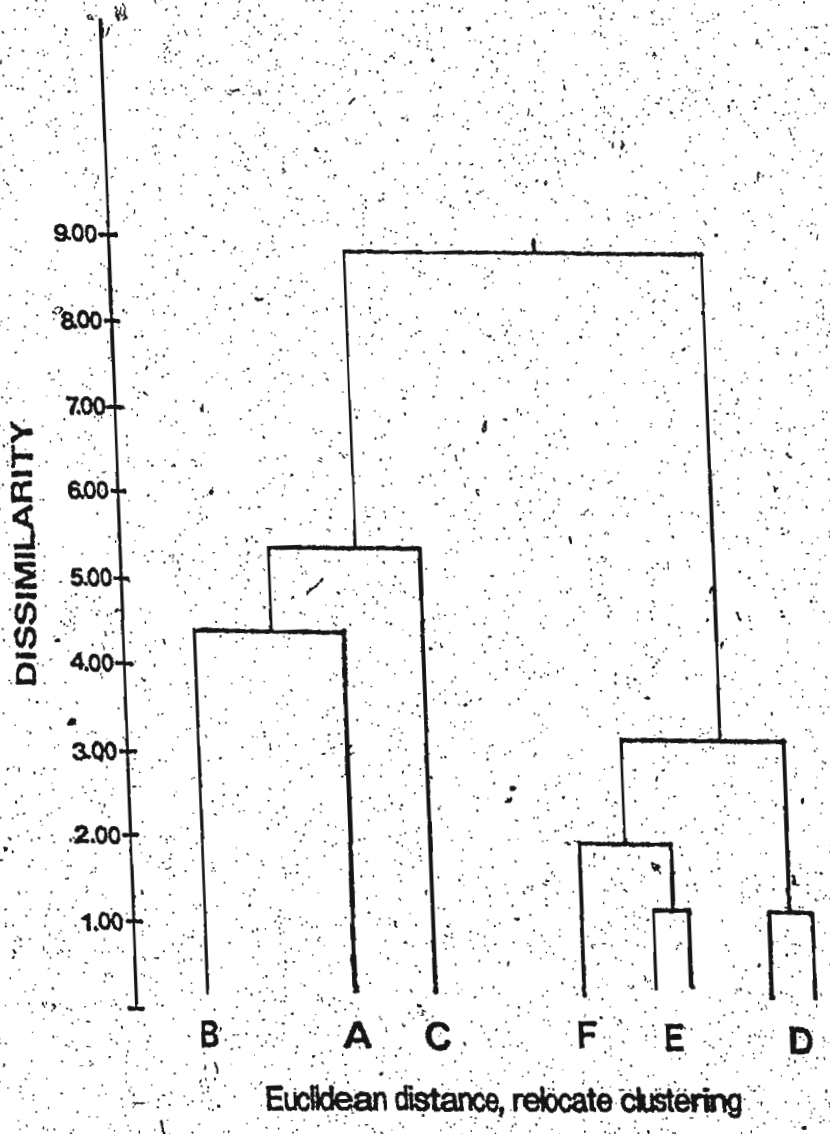

DISSIMILARITY
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
B
A
C
F
E
D
Euclidean distance, relocate clustering

Figure 10. Hierarchy of clustering produced by the Euclidean distance coefficient with Ward's clustering.

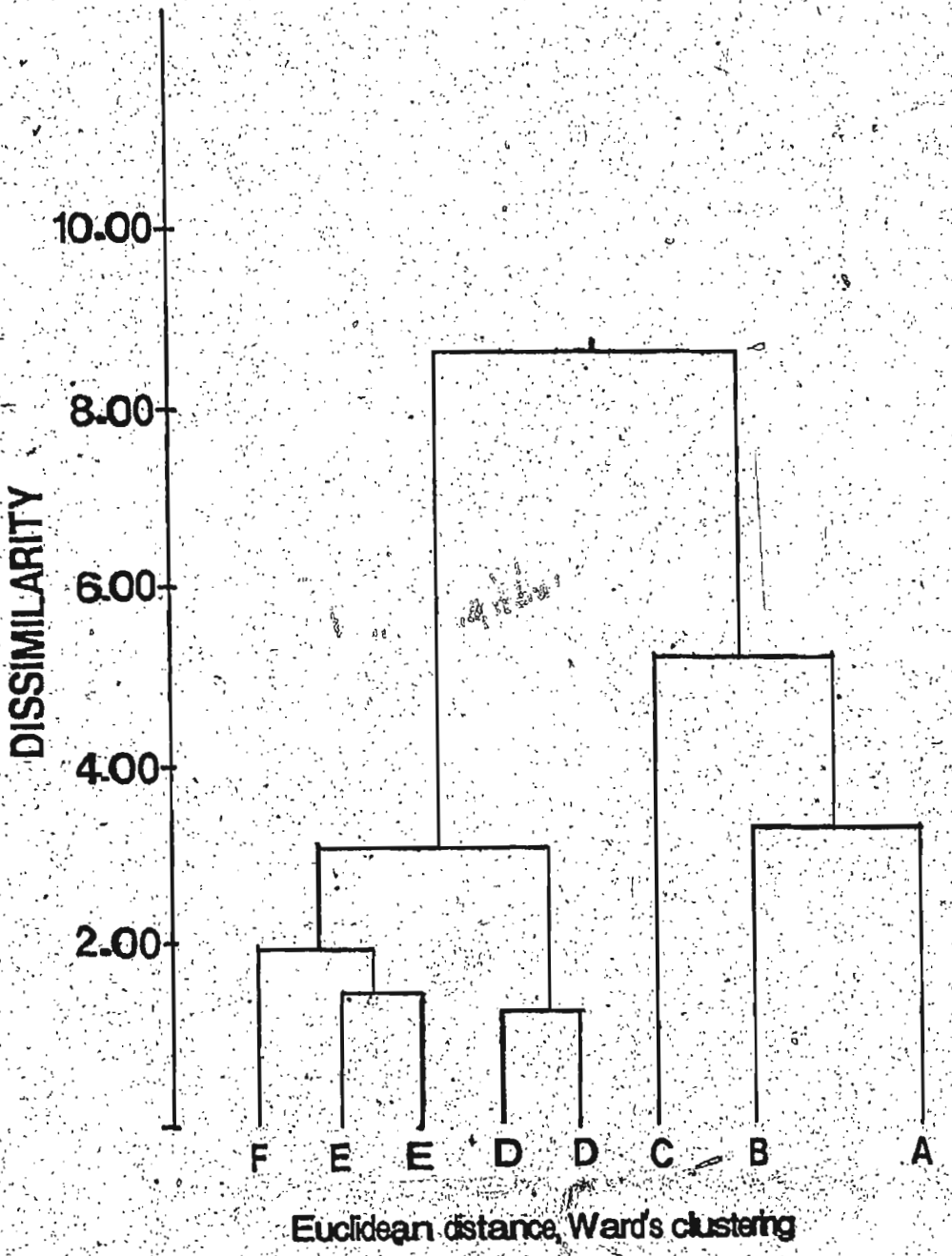
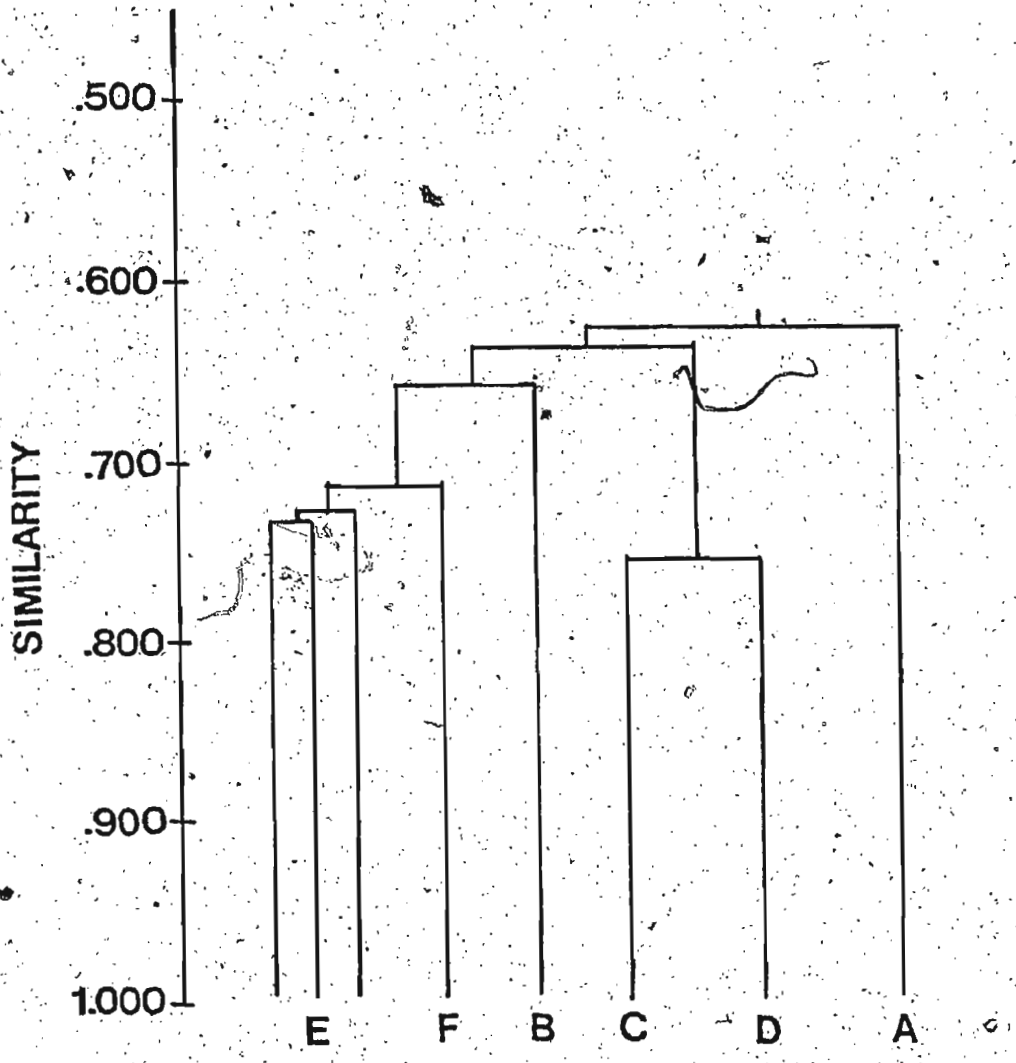
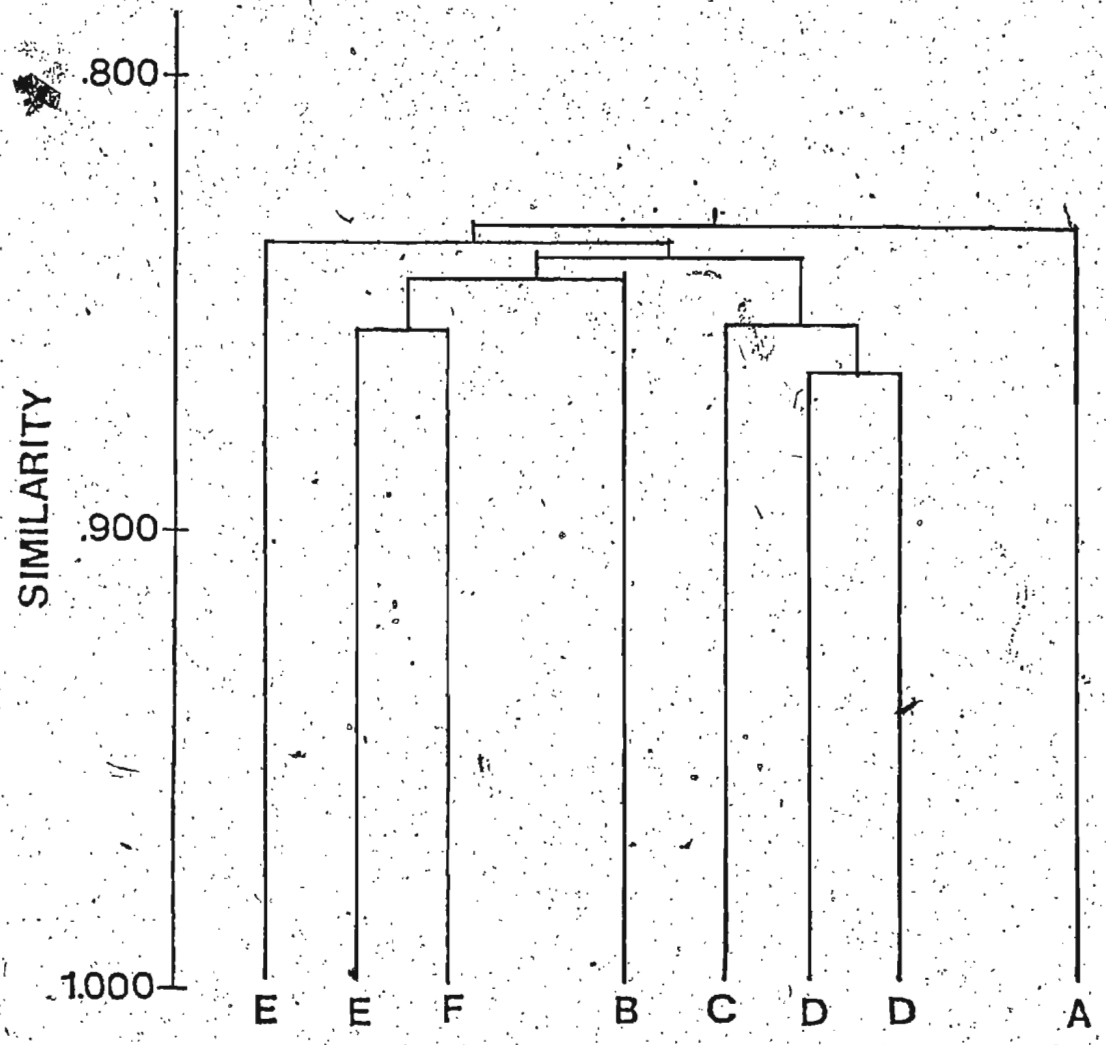

10.00
8.00
6.00
4.00
2.00
DISSIMILARITY
F
E
E
D
D
C
B
A
Euclidean distance, Ward's clustering

Figure 11. Hierarchy of clustering produced by the Jaccard coefficient with single linkage clustering.



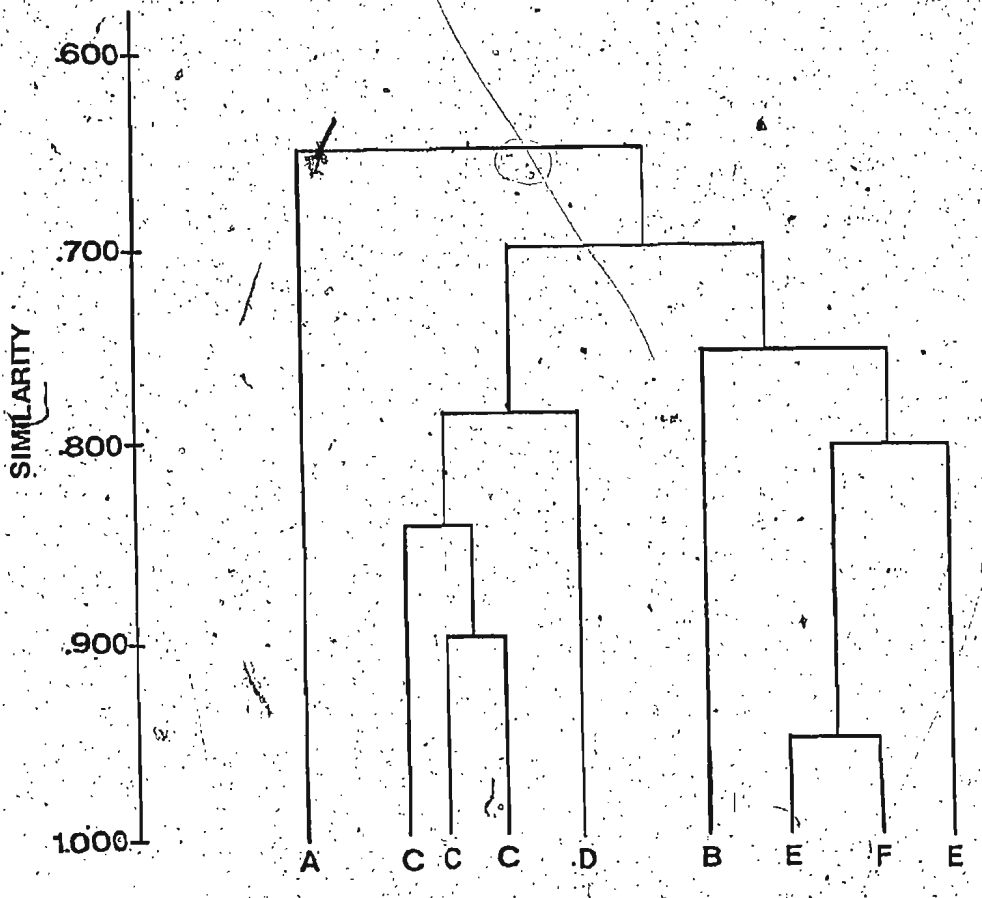
Jaccard coefficient, single linkage clustering

Figure 12. Hierarchy of clustering produced by the simple matching coefficient with single linkage clustering.



Simple matching coefficient, single linkage clustering

Figure 13. Hierarchy of clustering produced by the Jaccard coefficient with density clustering.



Jaccard coefficient, density clustering

linkage clustering were virtually the same. All three of these methods divided the clusters of OTU into three broad groups. The first group consists of clusters B, E and F. In all three dendrograms, cluster E was divided into subclusters of OTU. When the Jaccard coefficient with single linkage clustering was used, the subclusters of cluster E fused to form a single cluster which then fused with cluster F. However, when the simple matching coefficient was used, only part of cluster E fused with cluster F. The remaining subcluster of E fused later in the hierarchical process. Clusters E and F then fused with cluster B in all three hierarchies.

The second group of clusters defined by these three cluster analysis consisted of clusters C and D. These clusters fused, and the resulting C-D cluster fused to the large group formed by clusters B, E and F.

The third group of OTU defined by these methods consisted of cluster A. This is a distinct cluster of OTU which fuses with the group of organisms formed by the previous fusion of clusters B to F.

The hierarchical arrangements of the clusters obtained from the five cluster analysis programs were of two general patterns. One pattern was obtained from the Euclidean distance coefficient with either Ward's or relocate clustering. The other pattern was obtained from

the other three cluster analysis programs. The differences between the two patterns were minor. This may be interpreted as evidence that the OTU classification obtained from the five cluster analysis programs is robust (Wishart, 1978).

Characteristics of the clusters

Table 4 gives groups of OTU which clustered consistently by at least four out of the five cluster analysis methods used. There are six clusters, designated A to F, and 86% of the OTU from seaweed and scallops are in these clusters. The original source and the type of metabolism (oxidative or fermentative) of each strain within each cluster is given. Oxidative or fermentative metabolism is shown because, in traditional taxonomies, these are heavily weighted characters. For OTU from seaweed the date of isolation is given, i.e., whether they were isolated on June 13, 1979 (D1), August 15, 1979 (D2), or September 20, 1979 (D3).

It is apparent from Table 4 that the clusters conform to the source of the strains. The strains from seaweed clustered separately from the strains from scallops, with three clusters from each source. The majority of the strains from seaweed clustered according to the date of isolation and according to the oxidative or fermentative metabolism. Although there are some exceptions the strains

Table 4. List of OTU which cluster consistently in at least 4 out of 5 cluster analyses.

Cluster	Source*	Metabolism	OTU
A	seaweed; D1	fermentative	1,2,3,4,5,6,7,8,9,11,12,13,14,16, 17,20,21,22,23,24
B	scallop	fermentative	85,87,88,90,91,92,93,94,95,96,97, 98,109
C	seaweed; D2	fermentative	26,27,29,30,32,33,34,35,39,40,41, 42,43,44,45,46,47
	seaweed; D3	fermentative	48,49,50,51,52,53,54,55,56,57,58, 59,60,61,62,64,65
D	seaweed; D1	fermentative	10,15,18

* D1 strains were isolated on June 13, 1979.
D2 strains were isolated on August 15, 1979.
D3 strains were isolated on September 20, 1979.

Table 4 continued ...

Cluster	Source*	Metabolism	OTU
	seaweed; D2	fermentative	31,38
	seaweed; D2	oxidative	68,69,70,71,73,74,75,77,78,79
	seaweed; D3	oxidative	80,81,82
E	scallop	fermentative	115,116
	scallop	oxidative	117,118,120,127,128,129,130,131, 132,133,134,135,136,137,138,139
F	scallop	fermentative	99,100,101,102,103,104,105,106, 107,108,111
	scallop	oxidative	121,122,123,124,125,140

from scallops also clustered according to fermentative or oxidative metabolism.

On the general assumption that the clusters represent different populations of bacteria the following conclusions can be drawn: 1) The bacterial population of the scallop was different from that of the seaweed. 2) Furthermore, seaweed and scallops had different populations of oxidative and fermentative bacteria. 3) To some extent, the bacterial population of the seaweed changed with time during the degradation of the fronds.

The percentage frequency of positive characters found in clusters A to F is given in Table 5. This table helps to distinguish between those characters which are useful in distinguishing between the six major clusters and those which are of no real diagnostic value. Any character which occurs with approximately the same frequency in all clusters is not of any value in distinguishing clusters. Examples of this type of character are oxidase, catalase, luminescence, Voges-Proskauer, growth at 4°C, and utilization of L-glutamate, D-glucose, L-aspartate, succinate, fumarate, DL-lactate, pyruvate, L-proline, acetate, propionate, DL-malate, L-malate, D-arabinose, and hippurate. However, these characters may be useful for the identification of the small number of strains from scallop and seaweed which did not fall within the six major clusters. These OTU will be described later.

Table 5. Percentage ferquency of positive characters found in individual clusters.

Test	Cluster					
	A (20)*	B (13)	C (34)	D (18)	E (18)	F (17)
PHB accumulation	55.0	61.5	41.0	94.4	0	17.6
oxidase	65.0	100	100	94.4	94.0	100
catalase	25.0	84.6	74.3	94.4	83.0	94.1
arginine dihydrolase	0	0	85.7	5.6	11.0	0
denitrification	0	46.2	100	33.3	28.0	100
Voges-Proskauer	0	0	0	0	0	0
$NO_3 \rightarrow NO_2$	0	92.3	100	44.4	17.0	64.7
growth at 4°C	100	92.3	100	100	94.0	100
growth at 35°C	5.0	0	2.9	83.3	39.0	41.2
growth at 40°C	5.0	0	0	83.3	0	11.8
luminescence	0	0	0	0	0	0

* The number of strains in each cluster is given in parentheses.

Table 5 continued ...

Test	Cluster					
	A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
laminaranase	90.0	0	20.0	22.2	6.0	5.9
amylase	45.0	0	74.3	33.3	6.0	23.5
agar digestion	35.0	0	0	0	0	0
gelatinase	100	100	97.1	44.4	33.3	76.5
lipase	100	84.6	91.4	33.3	94.0	100
alginase	85.0	0	82.9	72.2	0	0
chitinase	15.0	15.4	5.7	0	0	5.9
cellulase (CMC)	85.0	0	0	0	0	0
D-ribose	5.0	0	100	94.4	11.0	0
D-xylose	60.0	7.7	0	5.6	11.0	5.9
D-arabinose	0	0	0	0	0	0
L-arabinose	5.0	0	0	0	39.0	23.5
sucrose	10.0	84.6	5.7	33.3	22.0	58.8

Table 5 continued ...

Test	Cluster A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
trehalose	65.0	0	97.1	61.1	28.0	23.5
maltose	85.0	76.9	100	55.6	17.0	70.6
cellobiose	20.0	23.1	100	88.9	0	0
melibiose	100	0	0	11.1	0	0
lactose	100	0	2.9	5.6	0	0
L-rhamnose	0	7.7	0	27.8	0	0
D-glucose	100	100	100	100	94.0	100
D-mannose	5.0	0	100	66.7	11.0	0
D-galactose	100	0	100	94.4	44.0	11.8
D-fructose	50.0	30.8	94.3	100	56.0	35.3
saccharate	0	7.7	0	0	22.0	70.6
mucate	0	7.7	0	0	28.0	58.8

Table 5 continued ...

Test	Cluster A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
D-galacturonate	0	0	0	16.7	39.0	88.2
L-glutamate	100	100	100	100	100	100
glycine	0	0	60.0	66.7	6.0	94.1
L-α-alanine	100	7.7	5.7	100	100	94.1
D-α-alanine	30.0	38.5	100	100	100	100
β-alanine	15.0	0	0	100	6.0	5.9
L-serine	5.0	76.9	97.1	88.9	61.0	100
L-leucine	15.0	100	2.9	100	72.0	100
L-isoleucine	100	0	0	50.0	0	5.9
DL-norleucine	5.0	0	0	38.9	0	0
L-valine	0	0	0	22.2	0	82.4
L-aspartate	15.0	100	100	100	89.0	100

Table 5 continued ...

Test	Cluster					
	A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
L-lysine	0	0	0	33.3	0	0
DL-citrulline	100	0	5.7	27.8	0	0
DL-α-aminobutyrate	0	0	0	0	0	11.8
γ-aminobutyrate	25.0	76.9	2.9	100	100	100
DL-α-aminovalerate	0	0	0	0	6.0	0
DL-arginine	100	0	94.3	100	0	5.9
DL-ornithine	5.0	0	2.9	100	28.0	94.1
N-acetyglucosamine	5.0	100	100	83.3	67.0	94.1
succinate	100	100	100	100	100	100
fumarate	100	100	100	100	100	100
DL-lactate	10.0	100	100	100	100	100
pyruvate	100	100	100	100	100	100

Table 5 continued ...

Test	Cluster A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
glycerol	25.0	0	100	100	50.0	100
L-proline	85.0	53.8	100	100	100	100
salicin	0	0	0	11.1	0	0
isobutyrate	0	0	0	27.8	72.0	94.1
isovalerate	0	0	0	94.4	61.0	88.2
malonate	0	0	2.9	94.4	61.0	88.2
DL-β-hydroxybutyrate	0	0	0	100	78.0	100
sorbitol	0	0	0	27.8	17.0	5.9
inositol	20.0	0	0	88.9	22.0	0
benzoate	0	53.8	0	0	33.3	0
p-hydrozybenzoate	70.0	0	0	11.1	56.0	17.6
phenylacetate	0	61.5	0	44.4	6.0	17.6

Table 5 continued ...

Test	Cluster					
	A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
quinate	0	0	0	44.4	50.0	23.5
δ-aminovalerate	5.0	0	0	33.3	56.0	17.6
betaine	30.0	0	2.9	100	33.3	94.1
sarcosine	15.0	0	2.9	100	28.0	76.5
hippurate	0	0	0	0	0	0
D-gluconate	100	7.7	82.9	100	44.0	100
D-glucuronate	15.0	0	40.0	38.9	44.0	100
valerate	30.0	100	0	100	72.0	100
DL-glycerate	5.0	76.9	9	100	50.0	100
α-ketoglutarate	5.0	100	100	100	100	100
propanol	0	0	2.9	88.9	0	94.1
L-threonine	0	0	100	27.8	0	0

Table 5 continued ...

Test	Cluster					
	A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
L-histidine	0	38.5	25.7	11.1	94.0	29.4
putrescine	0	0	0	27.8	0	0
maleate	0	0	0	33.3	0	5.9
adipate	0	0	0	27.8	0	17.6
suberate	0	0	0	0	83.0	94.1
azelate	0	0	0	0	72.0	100
sebacate	0	30.8	0	0	72.0	94.1
L-phenylalanine	5.0	0	0	38.9	50.0	82.4
L-tryptophan	0	0	0	0	33.3	17.6
n-butanol	0	0	0	27.8	6.0	70.6
itaconate	0	0	0	0	33.3	5.9
allantoin	0	0	0	27.8	0	5.9

Table 5 continued ...

Test	Cluster					
	A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
nicotinate	0	0	0	22.2	0	5.9
adonitol	0	0	0	0	6.0	23.5
glycolate	15.0	0	0	61.1	28.0	88.2
acetate	100	100	100	100	100	100
propionate	95.0	100	100	100	94.0	100
butyrate	0	100	2.9	77.8	77.8	100
heptanoate	0	0	0	88.9	100	76.5
pelargonate	75.0	0	0	83.3	33.3	29.4
ethanol	5.0	0	0	100	77.8	94.1
DL-malate	10.0	100	94.3	100	83.0	100
citrate	80.0	0	100	100	89.0	100
aconitate	15.0	0	100	100	89.0	100

Table 5 continued ...

Test	Cluster A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
mannitol	100	46.2	100	100	50.0	100
L-tyrosine	100	76.9	0	100	94.0	100
L-malate	100	100	100	100	100	82.4
fermentative breakdown of glucose	100	100	97.1	27.8	11.0	64.7
oxidative breakdown of glucose	0	0	2.9	72.2	89.0	35.3
aromatic ring cleavage	0	0	0	33.3	67.0	17.6

Characters which are of value in discriminating the six clusters are given in Table 6. These are characters possessed by at least 85% of the OTU in a cluster and occur with a binary frequencies ratio of at least 2.0. That is, the frequency of occurrence of each character in its respective cluster is at least twice as high as the frequency of occurrence in the entire set of six clusters.

A combination of the information in Tables 5 and 6 may be compiled to give a diagnostic table for the 6 major clusters (Table 7). Such a table can form the basis by which tests can be selected for the identification of the groups of marine eubacteria described in this study. The moles % G + C obtained by examining representative strains from each cluster are given in Table 8.

From information that has been given it is possible to describe each cluster. The OTU in all clusters were motile, gram-negative, rod-shaped bacteria which required Na^+ for growth. For each cluster a tentative identification of groups of OTU has been made at the generic level. A more detailed identification will be given in a later section.

Cluster A has 20 strains from seaweed, all of which were isolated on June 13, 1979. All are fermentative and grow at 4°C. Only one OTU could grow at 35°C and 40°C. Eleven strains accumulated PHB as an intracellular reserve when grown in broth containing DL-β-

Table 6. Characters which distinguish the individual clusters.

Cluster	Character Name	Character Category
A	cellulase (CM)	exoenzyme
	lactose	carbohydrate
	melibiose	carbohydrate
	DL-citrulline	aliphatic amino acid
	L-isoleucine	aliphatic amino acid
	laminaranase	exoenzyme
B	butyrate	fatty acid
	sucrose	carbohydrate
C	arginine dihydrolase	enzyme
	L-threonine	aliphatic amino acid
	D-mannose	carbohydrate
	D-ribose	carbohydrate
	cellobiose	carbohydrate
D	β-alanine	aliphatic amino acid
	growth at 40°C	growth temp.
	growth at 35°C	growth temp.
	inosol	polyalcohol/glycol
	propanol	alcohol

Table 6 continued ...

Cluster	Name	Category
D	sarcosine	misc. nitrogenous compound
	DL-ornithine	alphatic amino acid
	malonate	dicarboxylic acid
	betaine	misc. nitrogenous compound
	isovalerate	fatty acid
	pelargonate	fatty acid
	PHB accumulation	structural
	ethanol	alcohol
	DL-glycerate	hydroxyacid
	DL-β-hydroxybutryate	hydroxyacid
	D-ribose	carbohydrate
	heptanoate	fatty acid
	cellobiose	carbohydrate
E	suberate	carbohydrate
	L-histidine	aromatic amino acid
	heptanoate	fatty acid
F	L-valine	aliphatic amino acid
	D-galacturonate	carbohydrate
	azelate	dicarboxylic acid
	suberate	dicarboxylic acid
	propanol	alcohol

Table 6 continued ...

Cluster	Name	Category
	sebacate	dicarboxylic acid
	glycolate	hydroxyacid
	isobutyrate	fatty acid
	L-phenylalanine	aromatic amino acid
	DL-ornithine	aliphatic amino acid
	betaine	misc. nitrogenous compound
	D-glucuronate	carbohydrate
	malonate	dicarboxylic acid
	isovalerate	fatty acid
	glycine	aliphatic amino acid
	DL-glycerate	hydroxyacid
	DL-β-hydroxybutyrate	hydroxyacid
	ethanol	alcohol
	butyrate	fatty acid

Table 7. Some distinguishing properties of the six clusters.[a]

Test	Cluster A (20)[b]	B (13)	C (34)	D (18)	E (18)	F (17)
PHB accumulation	11	8	14	+	-	3
arginine dihydrolase	-	-	+	-	-	-
growth at 35°C	-	-	-	+	7	7
growth at 40°C	-	-	-	+	-	-
laminaranase	+	-	7	4	-	-
cellulase (CMC)	+	-	-	-	-	-
agar digestion	7	-	-	-	-	-
alginase	+	-	+	13	-	-
D-ribose	-	-	+	+	-	-
sucrose	-	+	-	6	4	10
maltose	+	10	+	10	-	12
cellobiose	4	3	+	+	-	-
melibiose	+	-	-	-	-	-
D-mannose	-	-	+	12	-	-
lactose	+	-	-	-	-	-
DL-citrulline	+	-	-	5	-	-
L-isoleucine	+	-	-	9	-	-

a: numbers indicate number of positive strains.

+ = at least 85% of the strains positive.

- = less than 15% of the strains positive.

b: Indicates the number of strains in each cluster.

Table 7 continued ...

Test	Cluster A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
L-threonine	-	-	+	5	-	-
β-alanine	-	-	-	+	-	-
L-histidine	-	5	9	-	+	5
DL-ornithine	-	-	-	+	5	+
L-valine	-	-	-	4	-	+
D-galacturonate	-	-	-	-	7	+
L-phenylalanine	-	-	-	7	9	+
D-glucuronate	-	-	-	+	6	+
glycine	-	-	20	12	-	+
inositol	4	-	-	+	4	-
α-ketoglutarate	-	+	+	+	+	+
propanol	-	-	-	+	-	+
putrescine	-	-	-	5	-	-
maleate	-	-	-	6	-	-
suberate	-	-	-	-	+	+
azelate	-	-	-	-	13	+
sebacate	-	4	-	-	13	+
heptanoate	-	-	-	+	+	13
citrate	16	-	+	+	+	+
L-tyrosine	+	10	-	+	+	+
aconitate	-	-	+	+	+	+

Table 7 continued ...

Test	Cluster A (20)	B (13)	C (34)	D (18)	E (18)	F (17)
itaconate	–	–	–	–	6	–
butyrate	–	+	–	14	14	+
ethanol	–	–	–	+	14	+
DL-glycerate	–	10	–	+	9	+
malonate	–	–	–	+	11	+
isovalerate	–	–	–	+	11	+
isobutyrate	–	–	–	5	13	+
DL-β-hydroxybutyrate	–	–	–	+	14	+
glycolate	–	–	–	11	5	+
sorbitol	–	–	–	5	3	–
betaine	6	–	–	+	6	+
glucose breakdown:						
oxidative	–	–	–	+	+	6
fermentative	+	+	+	–	–	11
flagella	PM[c]	PM	PM	PM	PM	PM

c: PM = polar monotrichous.

Table 8. Moles % G + C values obtained for representative strains.

Cluster	OTU	Moles % G + C
A	21	43.1
B	93	45.1
C	44	45.3
	59	45.3
D	18	45.3
	70	62.5
	71	62.5
	77	62.5
	79	62.5
	80	62.5
	81	62.5
E	118	44.4
F	122	43.1
	67	46.4
	133	62.5

hydroxybutyrate. None of them could luminesce, denitrify, produce acetoin and/or diacetyl, or possessed a constitutive arginine dihydrolase system. The strains in this cluster produced the most extracellular enzymes. There were some positive results for all eight exoenzymes tested and these were the only strains that produced cellulase and digested agar. Ninety percent of the strains produced laminaranase. This percentage was greater than that found in any other cluster. All strains produced gelatinase and lipase. The results of the nutritional screening showed that all of the strains were capable of growth on 17 of the 117 organic substrates tested. One or more of the strains were capable of utilizing 55 of the 117 compounds tested. The strain tested from this cluster had a moles % G + C of 43.1. The strains in this cluster can be identified as members of the genus Vibrio.

Cluster B contains 13 fermentative strains isolated from the scallop, P. magellanicus. Approximately two thirds of the strains accumulated PHB as an intracellular storage product when grown in broth containing DL-β-hydroxybutyrate. None of the strains luminesced, produced acetoin and/or diacetyl, or possessed a constitutive arginine dihydrolase system. Approximately half of the strains could denitrify. Twelve of the strains could grow at 4°C; none could grow at 35°C or 40°C. The only exoenzymes produced by the strains were gelatinase (100%),

lipase (84.6%), and chitinase (15.4%). The strains in cluster B were the least nutritionally versatile of all the isolates. Only 37 of the 117 organic compounds tested were utilized by one or more of the strains in this cluster. The strain tested in this cluster had a moles % G + C of 45.1. The strains in this cluster can be identified as members of the genus Vibrio.

Cluster C contains fermentative bacterial strains isolated from the seaweed A. esculenta. Seventeen of the strains were D2 isolates. The other seventeen strains were D3 isolates. All of the strains produced oxidase, denitrified, and grew at 4°C. None of the strains luminesced, produced acetoin and/or diacetyl, or were capable of growth at 40°C. Eighty-five percent of the strains in this cluster possessed a constitutive arginine dihydrolase, a far greater percentage than that found in any other cluster. Six of the seventeen D2 isolates in this cluster accumulated PHB as an intracellular storage product. These are OTU 31, 35, 36, 38, 40 and 41. OTU 31, 36 and 38 accumulated PHB when grown in broth containing DL-ß-hydroxybutyrate. OTU 35, 40 and 41 accumulated PHB when grown in broth containing D-glucose. Eight of the seventeen D3 isolates in this cluster accumulated PHB when grown in broth containing D-glucose. These were OTU 50, 51, 55, 56, 57, 60, 63 and 64. At least 75% of the isolates in this cluster were capable of producing an

extraceullular amylase, gelatinase, lipase and alginase. Twenty percent of the strains produced an extracellular laminaranase and 5.7% produced chitinase. None of the strains produced cellulase or agarase. Only 45 of the 117 organic compounds tested in the nutritional screening were utilized by one or more of the isolates. The strains tested in this cluster had a moles % G + C of 45.3. The strains in this cluster can be identified as belonging to the genus Vibrio.

Cluster D has 18 strains isolated from seaweed. Three of the strains, OTU 10, 15 and 18, are D1 isolates. OTU 31 and 38 are D2 isolates. All 5 of these strains are fermentative. The remaining 13 strains in this cluster are oxidative. OTU 68 to 71, 73 to 75, and 77 to 79 are D2 isolates. OTU 80 to 82 are D3 isolates. All strains grew at 4°C. All but one of the isolates in this cluster accumulated PHB, produced oxidase and catalase, and utilized D-ribose, D-galactose, isovalerate and malonate as sole sources of carbon and energy. All 17 of the strains which accumulated PHB did so when grown in broth containing the monomer, DL-β-hydroxybutyrate. Only one of the strains, OTU 31, possessed a constitutive arginine dihydrolase. None of the strains in this cluster luminesced, produced acetoin and/or diacetyl, digested agar, or produced an extracellular chitinase or cellulase. Strains in this cluster were nutritionally versatile, as

indicated by the fact that at least some of the strains utilized 79 of the 117 organic substrates tested as sole carbon and energy sources. Thirty-three of the compounds were utilized by 100% of the strains. Six of the oxidative strains in this cluster, OTU 77 to 82, were capable of aromatic ring cleavage. All 6 of these strains cleaved quinate via the ortho pathway. OTU 18, a fermentative strain, was found to have a G + C content in its DNA of 45.3 moles %. Therefore, the fermentative strains in cluster D can be identified as belonging to the genus Vibrio. Six oxidative strains, OTU 70, 71, 77, 79, 80 and 81, were found to have a G + C content in their DNA of 62.5 moles %. Therefore, the oxidative strains in cluster D can be identified as belonging to the genus Pseudomonas.

Cluster E has 18 strains isolated from scallop. Two of these strains, OTU 115 and 116, are fermentative. The remaining 16 strains are oxidative. This is the only cluster in which none of the strains accumulated PHB as an intracellular storage product. None of the strains luminesced, produced acetoin and/or diacetyl, grew at 40°C, digested agar, or produced an extracellular alginase, chitinase or cellulase. All but one strain grew at 4°C. With the exception of lipase, extracellular enzyme production was markedly low in this cluster. Lipase was produced by 94% of the strains. The results of the nutritional screening showed that only 10 of the 117 compounds tested were utilized by all of the strains.

This is a much lower number than that found in any other cluster. At least one positive result was obtained for 71 of the substrates. Eleven of the oxidative strains were capable of aromatic ring cleavage. OTU 117, 118, 120, 131, 132, 133 and 139 metabolized p-hydroxybenzoate via the ortho pathway. OTU 134 and 137 cleaved quinate via the ortho pathway. OTU 130 and 138 cleaved benzoate via the ortho pathway. The fermentative strains in this cluster may be tentatively identified as strains of the genus Vibrio. An oxidative strain from this cluster, OTU 118, was found to have a G + C content in its DNA of 44.4 moles %. Therefore, the oxidative strains in cluster E can be identified as belonging to the genus Alteromonas.

Cluster F has 17 strains, all of which were isolated from scallop. Eleven of the strains were fermentative and six were oxidative. Three of the strains accumulated PHB as an intracellular storage product when cultured in broth containing an excess of the monomer, DL-β-hydroxybutyrate. These are OTU 99, 100 and 101, which are fermentative. All of the strains produced oxidase, denitrified, produced an extracellular lipase, and grew at 4°C. None of the strains luminesced, produced acetoin and/or diacetyl, possessed a constitutive arginine dihydrolase, digested agar, or produced an extracellular alginase or cellulase. The strains in this cluster were nutritionally versatile. Twenty-eight of the organic

compounds tested were utilized by all of the strains as sole sources of carbon and energy. Binary results were obtained for 76 of the compounds. Three of the oxidative strains were capable of aromatic ring cleavage. These are OTU 121, 123 and 140, all of which metabolized p-hydroxybenzoate via the ortho pathway. An oxidative strain from this cluster, OTU 122, was found to have a moles % G + C content of 43.1. The fermentative strains in this cluster may be tentatively identified as members of the genus Vibrio, and the oxidative strains as members of the genus Alteromonas.

OTU which do not cluster consistently

The remainder of the seaweed and scallop isolates do not cluster consistently as a result of the five cluster analysis methods. These twenty OTU are listed in Table 9. They are a heterogenous collection of strains which, with a few exceptions, bear no relationship to each other or to any of the robust clusters.

It is interesting that this list includes OTU 66 and 67, the only two oxidative strains isolated from seaweed in the D1 set. These two OTU are closely related to each other, differing in only two of the one hundred and twelve characters studied, these being the utilization of L-α-alanine and α-ketoglutarate as sole carbon and energy sources. These two OTU probably represent a single bacterial species. They do not cluster closely to any of

Table 9. OTU which do not cluster consistently

OTU	Source	Metabolism
19	seaweed; D1	fermentative
25,28,36,37	seaweed; D2	fermentative
63	seaweed; D3	fermentative
66,67	seaweed; D1	oxidative
72,76	seaweed; D2	oxidative
83,84	seaweed; D3	oxidative
86,69,110,112,113,114	scallop	fermentative
119,126	scallop	oxidative
141	ATCC 29660	oxidative
142	ATCC 29659	oxidative
143	ATCC 27562	fermentative
144	ATCC 25914	fermentative
145	ATCC 27043	fermentative
146	ATCC 27126	oxidative
147	ATCC 25915	fermentative
148	ATCC 27125	oxidative
149	ATCC 27132	oxidative

the robust clusters. These strains are oxidative, and the only extracellular enzyme they produce is lipase. This, combined with their ability to utilize only 23 of the 117 organic compounds tested as sole carbon and energy sources, separates these two strains from the predominant seaweed and scallop flora on a phenotypic basis. OTU 67 was found to have a G + C content in its DNA of 46.4 moles %. This indicates that OTU 66 and 67 belong to the genus Alteromonas.

Other strains listed in Table 9 that are of interest are OTU 112, 113 and 114. These strains are closely related to each other, forming a tight group as a result of all five cluster analyses. All three OTU are fermentative and were isolated from scallop. The only exoenzymes produced by these strains are lipase and gelatinase. Thirty-eight organic compounds are utilized by at least one of the strains as sole carbon and energy sources. This is the same number as that found for the strains in cluster B, which are also fermentative strains isolated from scallop. Actually, twenty-eight of the substrates are common to both groups, and this is probably why OTU 112, 113 and 114 cluster near cluster B in three of the five cluster analyses.

Strains 83 and 84 are oxidative and both were isolated from seaweed with the D3 set. These strains are not related to any robust cluster and may represent a

single species since they differ from each other on a single character, this being the utilization of glycerol as a sole source of carbon and energy. Only 11 of the 117 organic compounds tested were utilized as sole sources of carbon and energy. Interestingly, these compounds are predominantly aliphatic amino acids and sugars. Hence, these strains are nutritionally isolated from any of the robust clusters. As a matter of fact, lack of nutritional versatility appears to be the main reason why the seaweed and scallop isolates listed in Table 9 do not cluster with any of the robust clusters.

The list of OTU in Table 9 also includes all nine reference strains included in the cluster analyses. Although there are a limited number represented it can be concluded that the type cultures, which are mesophilic, are different than the population of psychrophilic bacteria isolated from scallop and seaweed. This will be elaborated upon in a later section.

Exoenzyme production

As a point of general interest a synopsis of the results of the exoenzyme production tests for the seaweed and scallop strains is given in Table 10. The percentage of strains producing gelatinase, chitinase, and, to a lesser extent, lipase is about the same regardless of the source of the strains. However, among these three

Table 10. Percentage of isolates producing various exoenzymes.

	Source of OTU			
	Seaweed		Scallop	
Exoenzyme	Fermentative (65)*	Oxidative (19)	Fermentative (32)	Oxidative (24)
laminaranase	45	21	6	0
alginase	83	74	0	0
cellulase (CMC)	29	0	0	0
lipase	88	53	94	96
amylase	63	21	6	13
gelatinase	91	47	88	50
chitinase	9	0	9	0

* The number of OTU studied is given in parentheses.

exoenzymes, gelatinase and chitinase were produced by fermentative organisms more often than by oxidative organisms. More strains from seaweed produced laminaranase, alginase, cellulase and amylase, and twice as many fermentative strains from seaweed produced these exoenzymes than did oxidative strains from the same source. From Table 10 it is apparent that the source of the strains is more important than whether the strains are oxidative or fermentative with respect to the production of a variety of exoenzymes. However, among strains isolated from seaweed, the percentage of strains that produced exoenzymes was greater among fermentative strains than among oxidative strains.

Identification of OTU

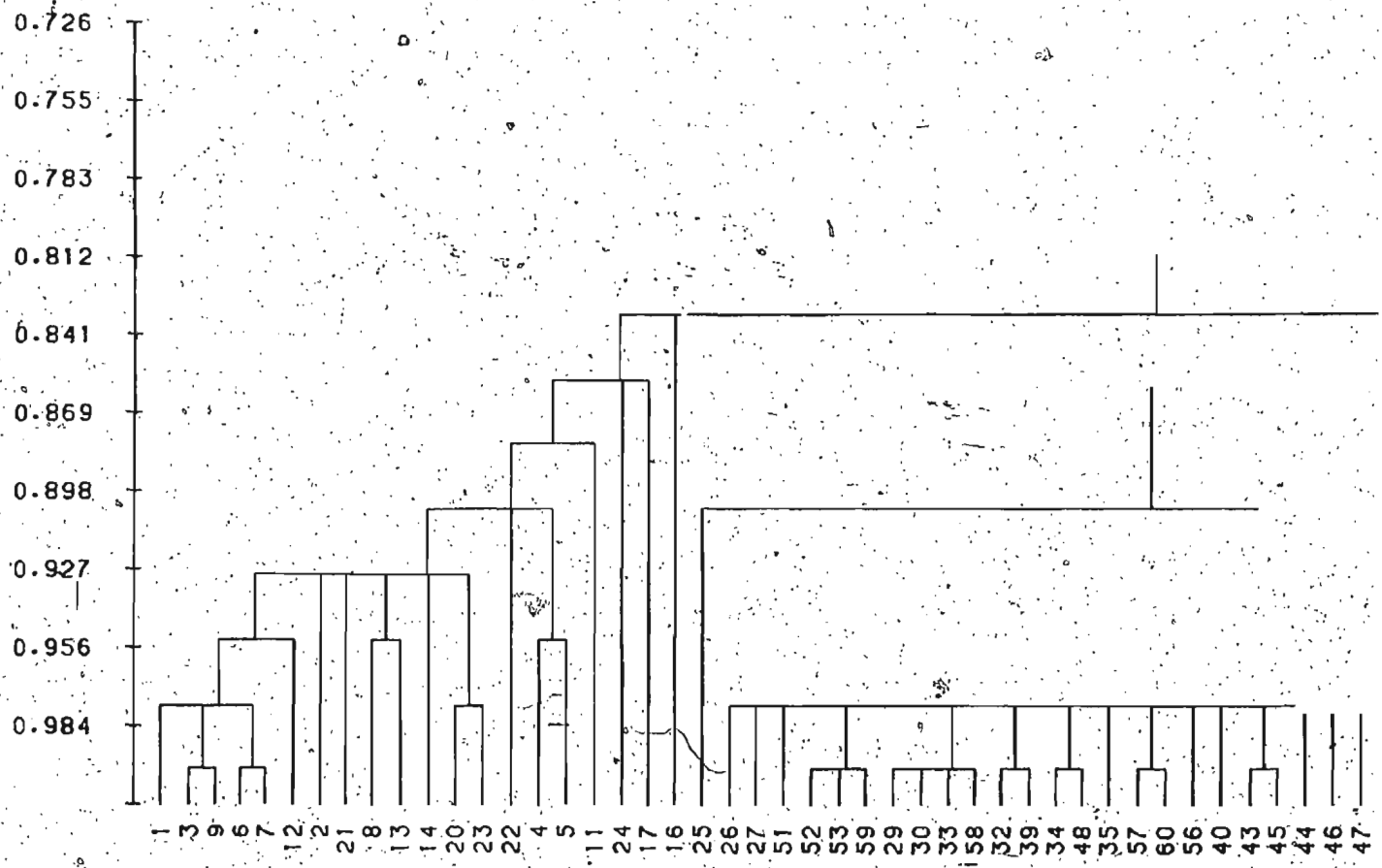
A data matrix which includes a large number of characters, such as the matrix used earlier in this study, is important for describing groups of organisms that have not been studied extensively. Cold ocean marine eubacteria are in this category. It requires a less extensive data matrix to determine if the cold ocean marine eubacteria can be identified by character sets that are known to be useful for the identification of mesophilic marine eubacteria. These character sets can be found in diagnostic tables published by Baumann and Baumann (1981).

The diagnostic tests used by Baumann and Baumann (1981) for the identification of mesophilic fermentative

marine bacteria are given in Appendix B, Table 1. The results of these tests for all of the fermentative strains from seaweed and scallop were compiled. In addition, results were compiled, from the literature, for all currently recognized type cultures of facultative marine eubacterial species. These results are given in a data matrix (Appendix B, Table 2) which was analyzed using the simple matching coefficient with single linkage clustering. The dendrogram which resulted from this analysis is given in Figure 14. It is apparent from Figure 14 that there are still six clusters, despite the use of a much smaller data set. Clusters A, B and C, and the fermentative component of clusters D, E and F are all intact. None of these clusters include any of the twenty-one reference strains. The latter strains form two distinct clusters containing species of Vibrio and Photobacterium.

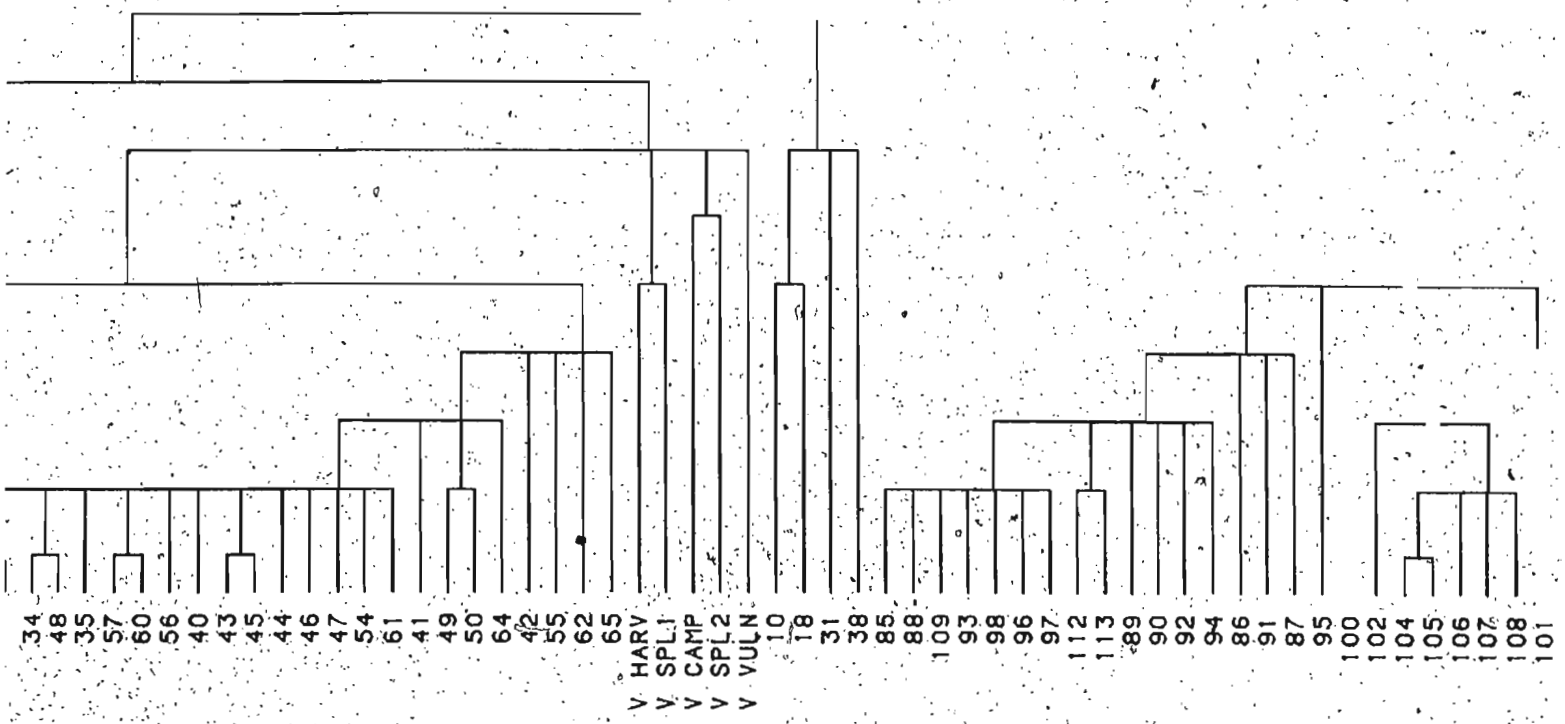
Cluster C, which contains fermentative strains from the D2 and D3 seaweed isolations, is a relatively tight cluster at the 90% similarity level. This cluster fuses with one of the Vibrio clusters at the 85% similarity level. Two representative strains from cluster C, OTU 44 (a D2 isolate) and OTU 59 (a D3 isolate), were examined using electron microscopy to confirm the type of flagellation. Both strains were confirmed as having a single polar flagellum when grown in liquid medium (Figures 15 and 16). The G + C content in the DNA of both OTU 44 and 59 was

Figure 14. Dendrogram produced by the analysis of the fermentative OTU using the simple matching coefficient with single linkage clustering. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.

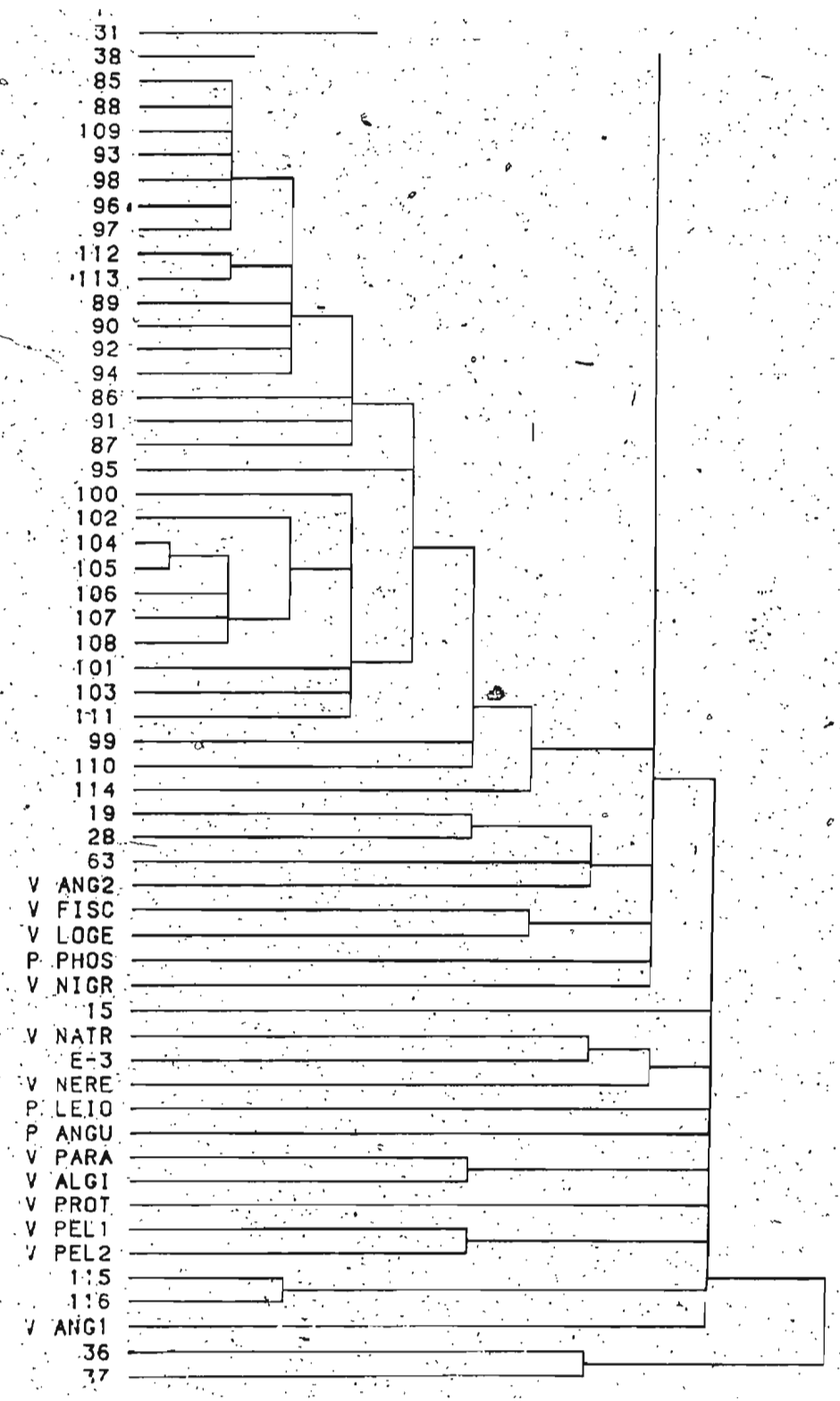


SIMPLE MATCHING COEFFICIENT SINGLE LINKAGE CLUSTERING

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V SPL1
V CAMP
V SPL2
V VULN
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V FISC
V LOGE
P PHOS
V NIGR
15
V NATR
E-3
V NERE
P LEIO
P ANGU
V PARA
V ALGI
V PROT
V PEL1
V PEL2
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V ANG1
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Figure 15. Electron micrograph of OTU 44 showing a single polar flagellum (34,100X).

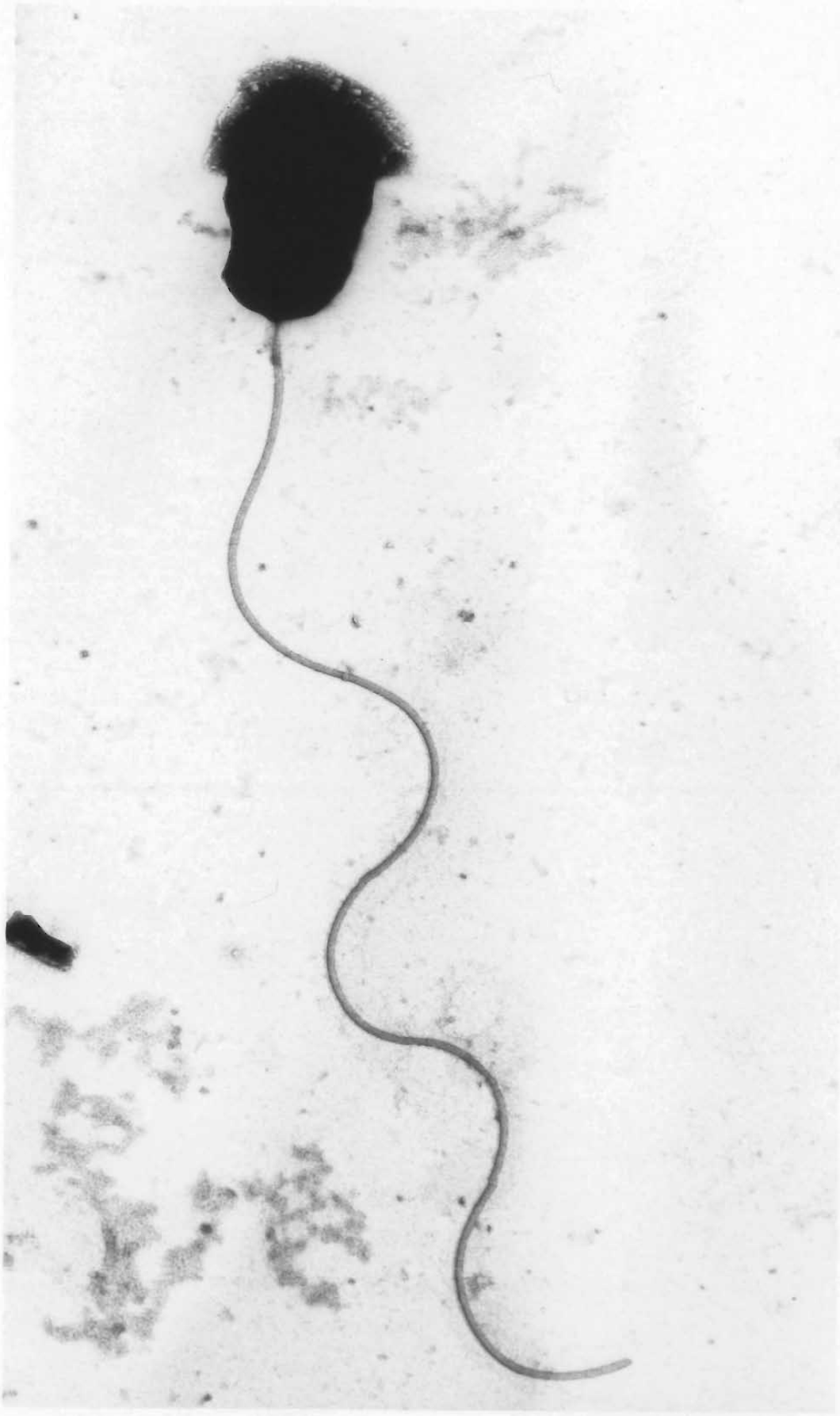
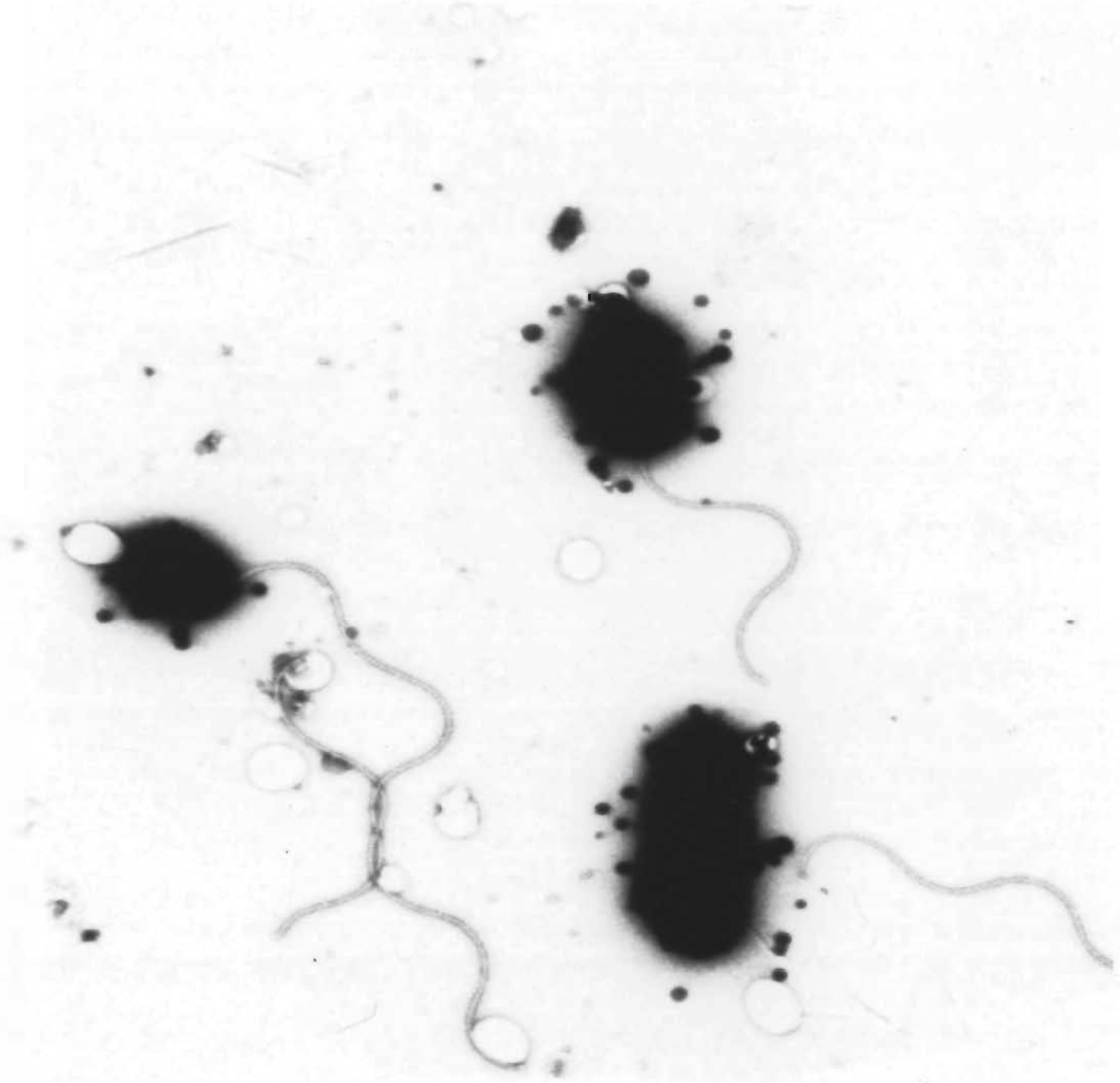


Figure 16. Electron micrograph of OTU 59 showing a single polar flagellum (12,600X).



found to be 45.3 moles %. Therefore, it may be concluded that the strains in cluster C are members of the genus Vibrio, and may represent a new species.

Cluster A, which contains fermentative strains from the D1 seaweed isolation, joins the C-Vibrio cluster at the 83% similarity level. OTU 21, a representative strain from cluster A, was found to have a G + C content in its DNA of 43.1 moles %. It was confirmed as having a single polar flagellum when grown in broth and observed using electron microscopy (Figure 17). Therefore, it can be concluded that the strains in cluster A belong to the genus Vibrio, and they may represent a new species.

Cluster B and the fermentative component of cluster F fuse at the 87% similarity level and these, in turn, fuse with the A-C-Vibrio cluster at the 79% similarity level. Here they are joined by 4 of the OTU which comprise the fermentative component of cluster D. OTU 93, a representative strain from cluster B, was found to have a single polar flagellum when grown in broth and examined using electron microscopy (Figure 18). It had a G + C content in its DNA of 45.1 moles %. Therefore, the strains from cluster B, which were isolated from scallop samples, can be identified as belonging to the genus Vibrio, and they also may represent a new species. Based on phenotypic characters only, the fermentative strains from cluster F can also be identified as belonging to the genus Vibrio,

Figure 17. Electron micrograph of OTU 21 showing a single polar flagellum (40,300X).

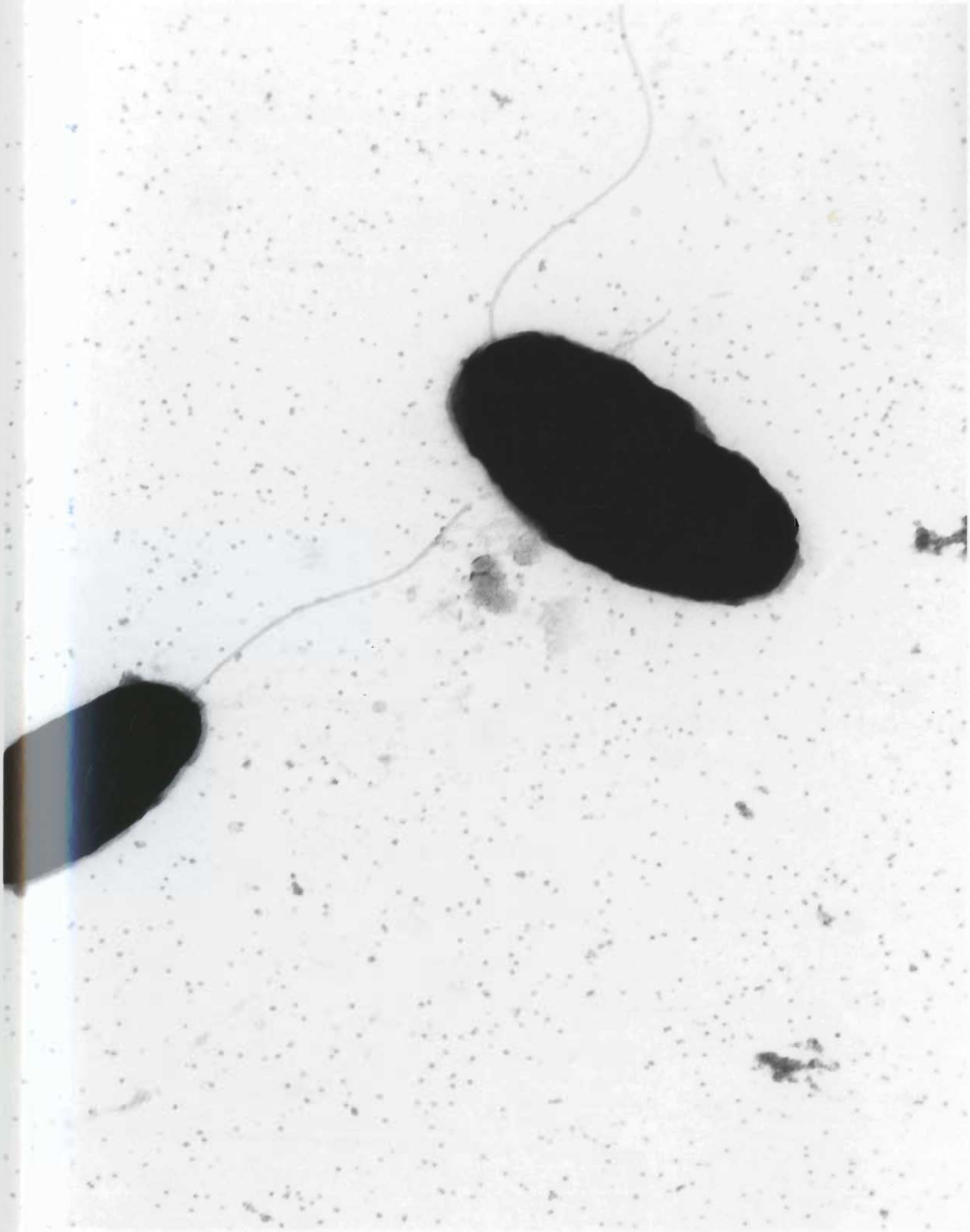
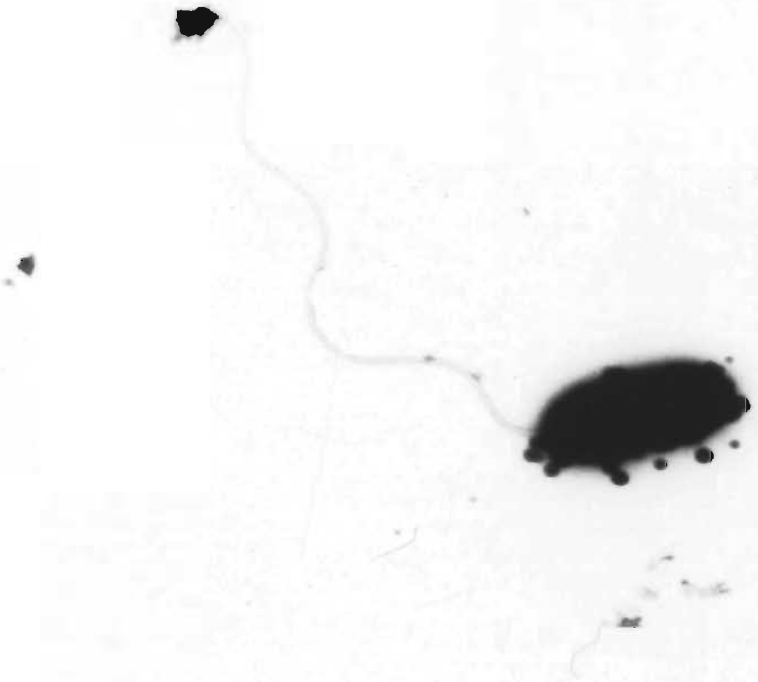


Figure 18. Electron micrograph of OTU 93 showing a single polar flagellum (12,600X).



and they may represent a species not yet described in the literature.

All of the fermentative strains in cluster D were isolated from seaweed during the D1 and D2 isolations. OTU 18, a D1 isolate, was found to have a G + C content in its DNA of 45.3 moles %. This is consistent with the genus Vibrio. Therefore, the fermentative strains from cluster D can be assigned to the genus Vibrio. These also may represent a new species.

The two fermentative strains from cluster E, OTU 115 and 116, occur within the larger cluster of mesophilic type strains. Based on phenotypic characters already described, OTU 115 and 116 can be tentatively identified as belonging to the genus Vibrio. A more definite identification can only be made when data on the moles % G + C of these strains are obtained.

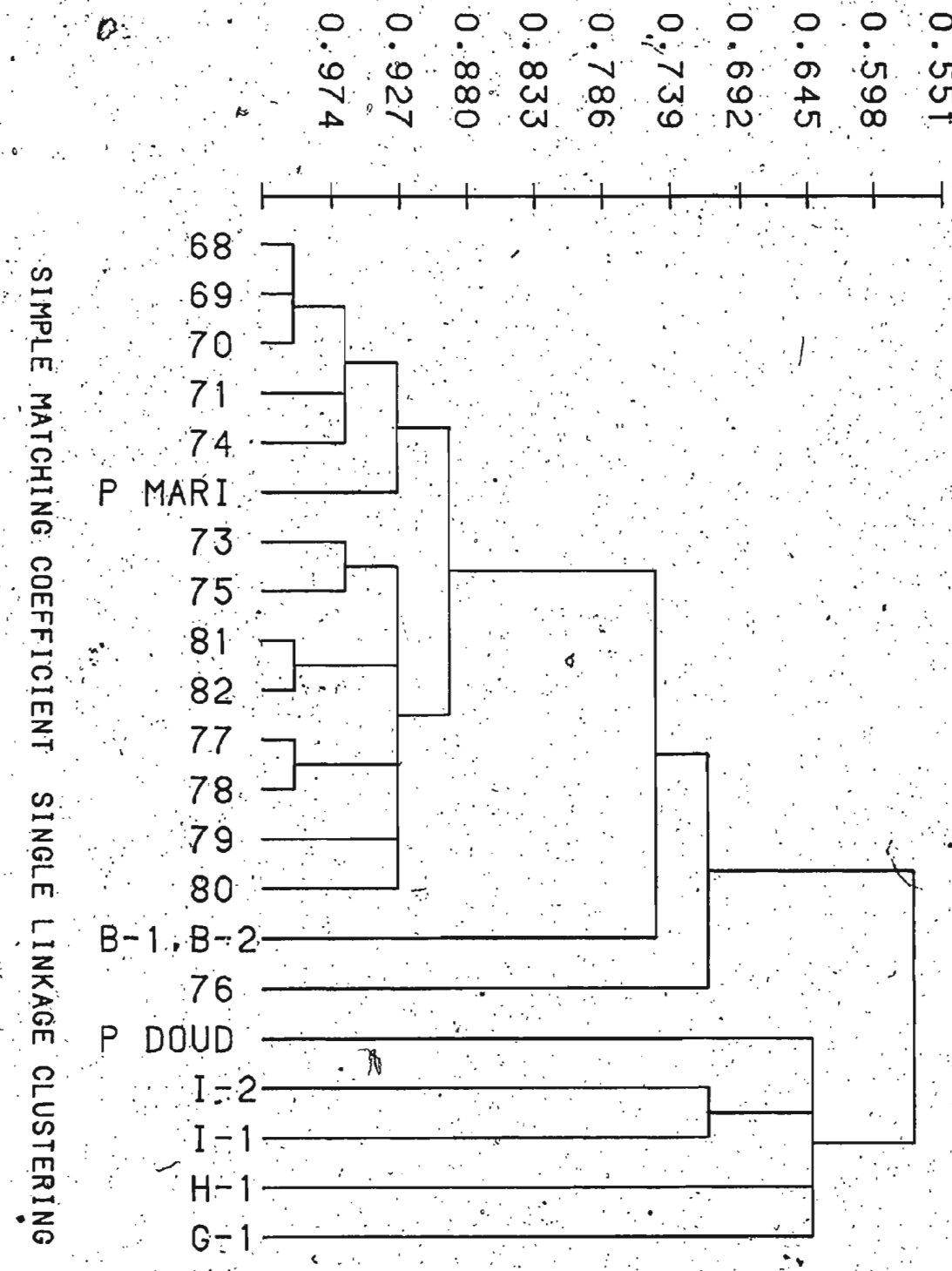
OTU 19, 28 and 63, which do not occur in clusters A to F, form a small cluster in this analysis at the 83% similarity level with Vibrio anguillarum biotype II and may be tentatively identified as strains of this species.

The diagnostic tests used for the identification of oxidative marine bacteria which accumulate PHB are given in Appendix B, Table 3. The results of these tests for all of the oxidative, PHB-accumulating strains from seaweed, as well as the reference strains listed in Baumann and Baumann (1981), were compiled into a data matrix which is given in

Appendix B, Table 4. The data were analyzed using the simple matching coefficient with single linkage clustering. None of the oxidative strains from scallop accumulated PHB. The dendrogram which resulted from this analysis is given in Figure 19. All except one of the oxidative, PHB-accumulating strains from seaweed are in cluster D. The remaining strain, OTU 76, is not a member of a particular cluster. It can be seen from the dendrogram (Figure 19) that the oxidative OTU of cluster D form a distinct cluster at the 88% similarity level which includes Pseudomonas marina. OTU 70, a representative strain from this cluster, was confirmed as having a single polar flagellum when grown in broth and observed using electron microscopy (Figure 20). OTU 70, 71, 77, 79, 80 and 81 were found to have a G + C content in their DNA of 62.5 moles %. This is within the range of values published for P. marina (Baumann and Baumann, 1981). Therefore, it can be concluded from phenotypic and genetic evidence that the oxidative OTU in cluster D are strains of P. marina. OTU 76 has not been identified.

The diagnostic tests used to identify oxidative marine bacteria which do not accumulate PHB are given in Appendix B, Table 5. The results of these tests for the seaweed and scallop strains, which are oxidative and do not accumulate PHB, as well as the appropriate reference strains listed in Baumann and Baumann (1981), were compiled into a data matrix (Appendix B, Table 6) which was analyzed using

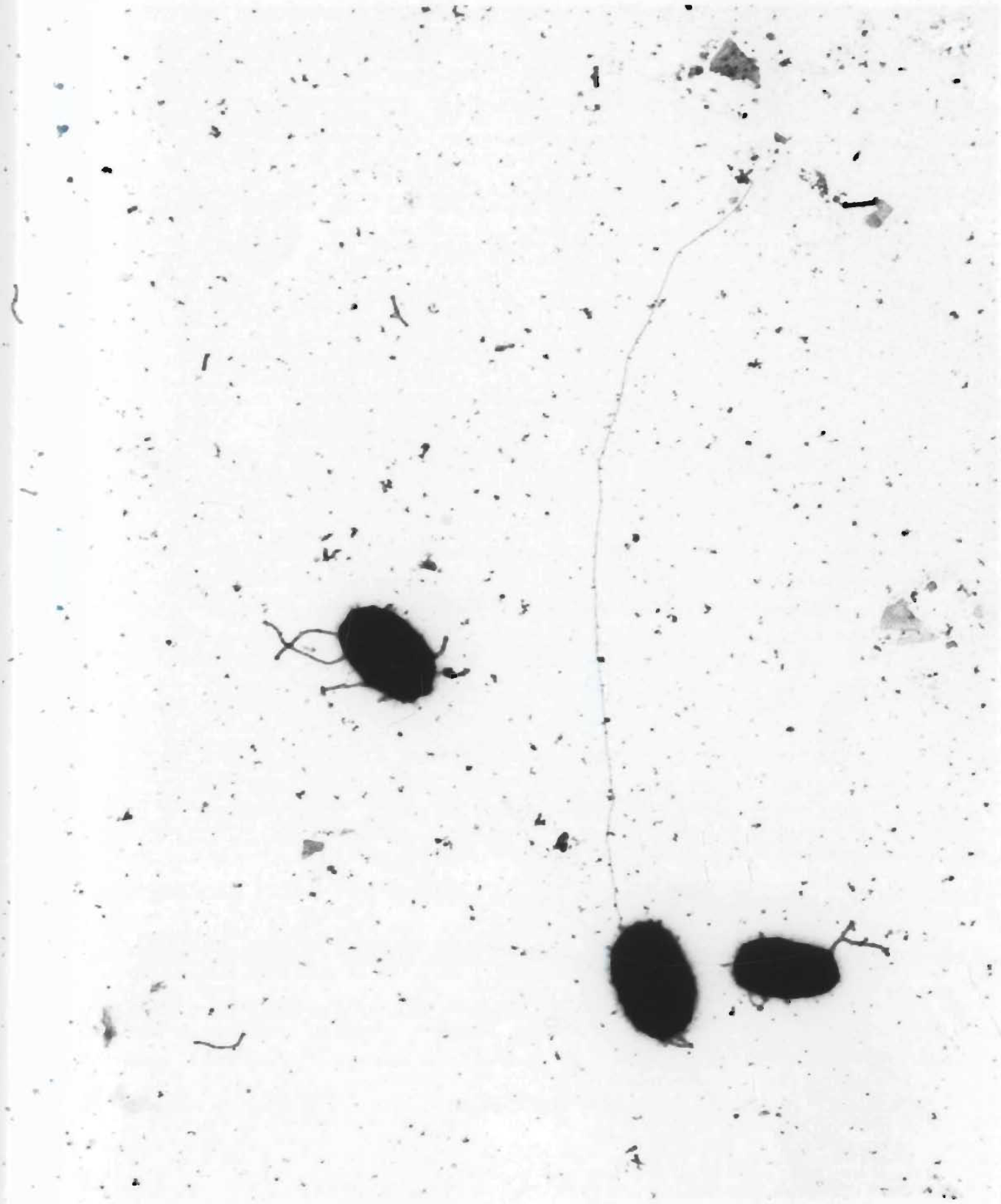
Figure 19. Dendrogram produced by the analysis of the oxidative, PHB-accumulating strains using the simple matching coefficient with single linkage clustering. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.



SIMPLE MATCHING COEFFICIENT SINGLE LINKAGE CLUSTERING

Figure 20. Electron micrograph of OTU 70 showing a single polar flagellum (12,600X).



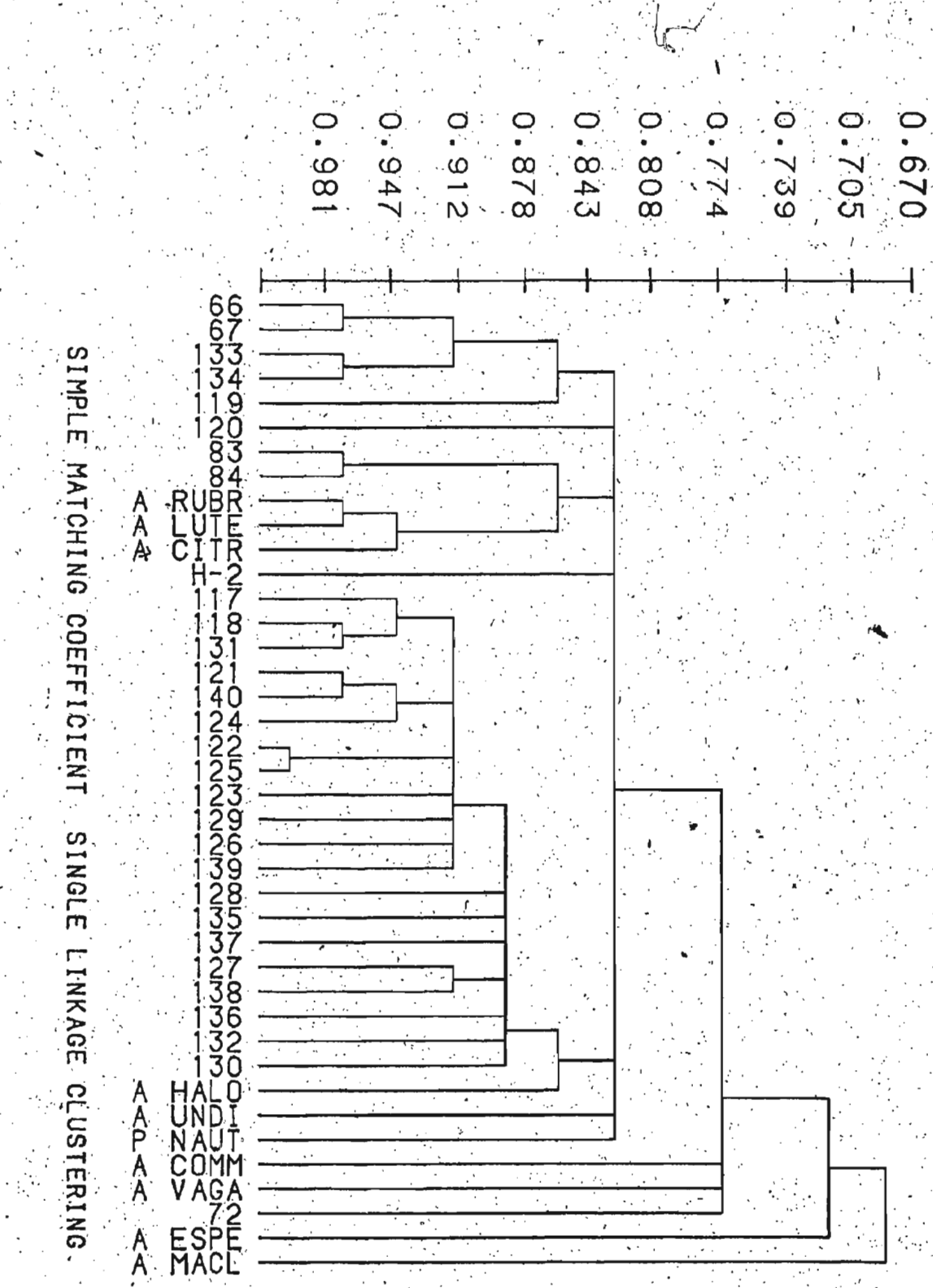


the simple matching coefficient with single linkage clustering. The resulting dendrogram is given in Figure 21.

Only five of the oxidative strains from seaweed do not accumulate PHB. These strains are not members of any particular cluster and likewise do not form a distinct cluster in this analysis. Three of these strains, OTU 72, 83 and 84 appear to be associated with species of Alteromonas, but even a tentative identification is not possible. The remaining two strains, OTU 66 and 67, the only D1 (seaweed) oxidative strains in the entire study, fuse with OTU 133 and 134 at the 91% similarity level. The latter two OTU are scallop isolates from cluster E. This group of four OTU cannot be identified at the species level. However, OTU 67 was found to have a G + C content in its DNA of 46.4 moles %, indicating that OTU 66 and 67 belong to the genus Alteromonas. OTU 133 was found to have a G + C content in its DNA of 62.5 moles %, indicating that OTU 133 and 134 belong to the genus Pseudomonas. OTU 119 and 120 cannot be identified at the present time.

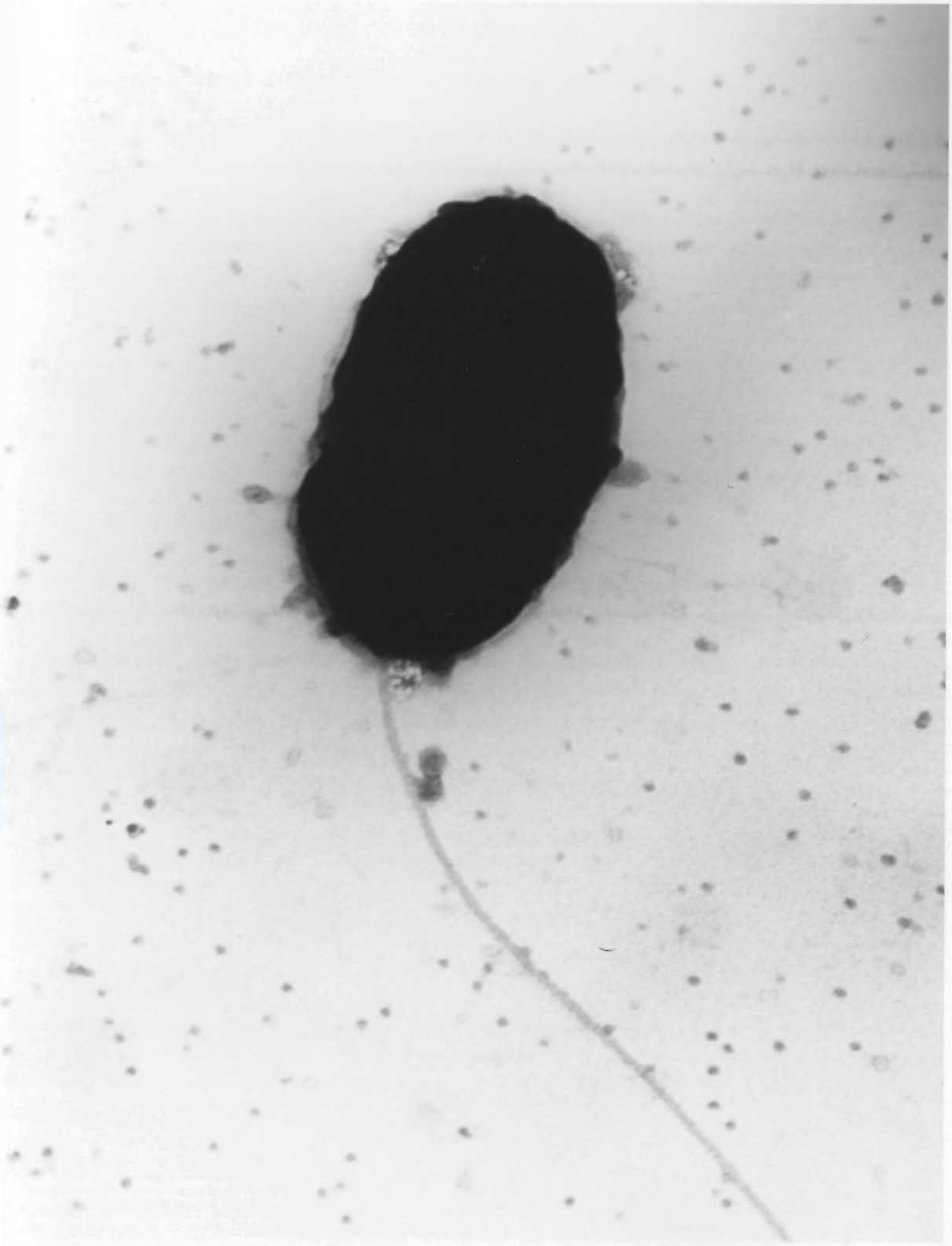
The remaining OTU in this analysis are oxidative strains from scallop. They belong to clusters E and F. It can be seen from the dendrogram (Figure 21) that cluster E is fragmented. All of the oxidative OTU in cluster E, except the two mentioned earlier, form a large cluster at the 87% similarity level which also includes the oxidative OTU from cluster F. The OTU from cluster F divide the OTU

Figure 21. Dendogram produced by the analysis of the oxidative, PHB-negative strains using the simple matching coefficent with single linkage clustering. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.


SIMPLE MATCHING COEFFICIENT SINGLE LINKAGE CLUSTERING.
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A RUBR
AA LUTE
A CITR
H-2
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137
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138
136
132
130
A HALO
AA UNDI
P NAUT
A COMM
A VAGA
72
A ESPE
A MACL

from cluster E into two subclusters. According to the dendrogram this large cluster of oxidative, PHB-negative marine bacteria form a cluster with Alteromonas haloplanktis at the 85% similarity level, and therefore may be identified as strains of this species. However, eleven of the OTU from this cluster are capable of aromatic ring cleavage by the ortho pathway. This characteristic is not possessed by type cultures of A. haloplanktis. A representative strain from the OTU that were not capable of aromatic ring cleavage, OTU 122, was found to have a G + C content in its DNA of 43.1 moles %. This is within the range of values that have been published for A. haloplanktis (Baumann and Baumann, 1981). OTU 122 was also confirmed as having a single polar flagellum when it was grown in broth and observed using electron microscopy (Figure 22). Therefore, the strains which are not capable of aromatic ring cleavage may be identified on a phenotypic and genetic basis as strains of Alteromonas haloplanktis. A representative strain which was capable of aromatic ring cleavage, OTU 118, was found to have a G + C content in its DNA of 44.4 moles %. This is only slightly greater than the highest published value for A. haloplanktis of 44.0 moles % (Baumann and Baumann, 1981). Therefore, the strains in this group which are capable of aromatic ring cleavage can be identified as either a new biotype of A. haloplanktis or possibly a new species of Alteromonas.

Figure 22. Electron micrograph of OTU 122 showing a single polar flagellum (86,970X).



In summary, it can be stated that the fermentative strains from each of the six major clusters cannot be identified at the species level, but they may represent new species in the genus Vibrio, a separate species being represented by each cluster. Three fermentative strains which do not cluster consistently, OTU 19, 28 and 63, can be tentatively identified on a phenotypic basis as strains of V. anguillarum biotype II. All three OTU were isolated from seaweed, each on a different sampling date. The oxidative strains from cluster D, all of which accumulated PHB and were isolated from seaweed (D2 and D3), can be identified on a phenotypic and genetic basis as strains of Pseudomonas marina. The only two oxidative strains from the D1 seaweed isolation, OTU 66 and 67, were identified as belonging to the genus Alteromonas and they may represent a new species. The oxidative strains from clusters E and F (except OTU 120, 133 and 134), which were isolated from scallop and did not accumulate PHB, can be identified on a phenotypic and genetic basis as strains of Alteromonas haloplanktis and, in the case of those strains which were capable of aromatic ring cleavage via the ortho pathway, either a new biotype of A. haloplanktis or a new species of Alteromonas. OTU 133 and 134 were identified as belonging to the genus Pseudomonas and they may represent a species not yet described.

The identification of the strains in clusters A to F is summarized in Table 11. The information given in this table also augments the assertion, made earlier, that different species of bacteria were associated with the seaweed and the scallop and that, to an extent, the species of bacteria on the seaweed changed with time.

Table 11. Identification of the major clusters of OTU obtained in this study.

Cluster	Source	Genus/species*
A	seaweed; D1	possible new species of *Vibrio*; designated *Vibrio* sp. a.
B	scallop	possible new species of *Vibrio*; designated *Vibrio* sp. b.
C	seaweed; D2, D3	possible new species of *Vibrio*; designated *Vibrio* sp. c.
D	seaweed; D1, D2	possible new species of *Vibrio*; designated *Vibrio* sp. d.
	seaweed; D2, D3	*Pseudomonas marina*

* Identification at the species level was carried out when possible using the criteria of Baumann and Baumann (1981).

Table 11 continued ...

Cluster	Source	Genus/species
E**,F	scallop	possible new species of Vibrio; designated Vibrio sp. e and Vibrio sp. f.
	scallop	Alteromonas haloplanktis and either a new biotype of A. haloplanktis or a new species of Alteromonas.

** Except OTU 120, 133 and 134.

DISCUSSION

For this study, the methods of numerical taxonomy were used to classify cold ocean bacteria from two sources. These were seaweed (Alaria esculenta) and giant scallop (Placopecten magellanicus). The principal objective of this study was to determine if cold ocean marine bacteria are the same species as their mesophilic counterparts from temperate waters, but with a lower optimum temperature for growth, or whether they actually constitute different species. A. esculenta and P. magellanicus were chosen as the sources of the strains because some earlier work had been done to characterize the strains from these sources (Hollohan, 1980; Powell, 1978) and the preliminary results indicated that there might be several different types of bacteria present. Also, a review of the literature showed that the bacterial flora of these organisms had not been studied in depth.

The strains studied were from a cold ocean environment and the majority of the strains were obtained when the water temperature was approximately 5°C. Although the psychrophilic nature of the strains was not determined in this study it was observed that almost all of the strains grew well at 4°C. A minority of the strains studied by Baumann and Baumann (1981) grew at 4°C. Studies have shown that bacteria isolated from the coastal waters of Newfoundland and from the Grand Banks of Newfoundland have an optimum temperature for growth of 5°C to 12.5°C

and that they grow well at 1°C (Gow, personal communication; Mills, B.Sc. Honours Thesis, Memorial University of Newfoundland, in preparation). This type of growth response with temperature is typical of psychrophiles (Morita, 1975).

Although oxidative and fermentative metabolism are heavily weighted characters in traditional taxonomies, and oxidative bacteria are often studied separately from fermentative organisms, these two types of organisms were included together in the numerical analyses performed in this study to determine whether the clusters which resulted would reflect the natural groupings of the strains. For all but a few exceptions this turned out to be the case. However, oxidative and fermentative strains were separated in the cluster analyses which compared the psychrophilic strains with the mesophilic type strains listed in Baumann and Baumann (1981). This was done because it would have been redundant to include both fermentative and oxidative bacteria together in such analyses.

The principles of numerical taxonomy are well established, and several hundred publications concerning the application of these techniques to bacterial classification and identification have been published during the last 25 years (Colwell and Austin, 1981). The most commonly used coefficients in such studies have been the simple matching and Jaccard coefficients. In view of this, Austin and Colwell (1977) evaluated a total of 31 coefficients for use in numerical taxonomy of microorganisms. These authors

found that 15 of these coefficients provided useful discriminating properties. They concluded that a wide range of coefficients can be useful in numerical taxonomy of bacteria, and that the choice of coefficient need not be limited to the simple matching or Jaccard coefficient. These authors also recognized, however, that the choice of coefficient remains subjective. Researchers have continued to use either the simple matching or Jaccard coefficient, perhaps because these coefficients are relatively simple, and therefore easy to understand (Sneath and Sokal, 1973). A few examples of papers which have employed the simple matching coefficient are Baumann et al. (1971a), Baumann et al. (1972), Pfister and Burkholder (1965) and Gillespie (1981). The Jaccard coefficient has also been used in many studies, including Kaneko et al. (1977, 1979), Hauxhurst et al. (1980) and Atlas et al. (1982). For reasons which have already been mentioned in the Introduction, Colwell and Austin (1981) have recently recommended that numerical taxonomic studies of bacteria utilize both the simple matching and Jaccard coefficients. This practice has been followed by Colwell and coworkers, as well as others, for some time (Austin et al., 1979; Sochard et al., 1979; Gray and Stewart, 1980). The study reported in this thesis utilized the simple matching and Jaccard coefficients, both with single linkage clustering. The resulting classifications were virtually identical, as was the case with the studies mentioned above. This indicates that the exclusion

of negative matches, as with the Jaccard coefficient, had minimal, if any, effect on the classification which was produced.

Three additional cluster analysis programs were used in this study to test the robustness of the OTU classification. The classifications produced by these three programs coincided. In fact, the classifications produced by the Euclidean distance coefficient with both Ward's and relocate clustering were identical. The classification produced by the Jaccard coefficient with density clustering was similar to that produced by the simple matching and Jaccard coefficients with single linkage clustering. That is, the hierarchical arrangements of the clusters obtained from the five cluster analyses were of two general patterns. One pattern was obtained when the Euclidean distance coefficient was used, the other with the simple matching or Jaccard coefficient. Hence, the differences in clustering patterns result from using either a dissimilarity or similarity measure. However, the differences between the two general hierarchical patterns were minor. Even more importantly, the OTU in the major clusters varied little, if at all, between analyses. This indicates that the OTU classification obtained in this study is taxonomically robust when tested using the criteria of Wishart (1978). A review of the pertinent literature indicates that the study reported in this thesis is the first in which the

criteria of Wishart (1978) were applied in testing the robustness of the OTU classification for bacteria.

This study also represents one of the first known attempts to apply the taxonomic schemes devised by Baumann and Baumann (1981) for the characterization of the bacterial flora of macroscopic marine organisms, specifically seaweed and giant scallops. Bacteria isolated from marine sources have been characterized in many studies conducted during the past two decades. These studies have employed either the original taxonomic scheme of Shewan et al. (1960) or more recent revisions of that scheme such as those published by Gibson et al. (1977) and Sochard et al. (1979). In all cases the strains were identified at the genus level. In most cases only a limited number of strains were isolated, often from many sources. This makes it difficult to observe any correlation between source of strains and groups identified.

Colwell and Liston (1962a) studied the natural bacterial flora of several marine invertebrates as part of a general ecological survey of marine commensal bacteria. The invertebrate samples included Crassostrea gigas (Pacific oyster), Halichondria panicea (marine sponge), Loligo opalescens (squid), Metridium senile (sea anemone), Aurelia sp. (medusa), and Pseudostylochus ostreophagus (flatworm) which were collected in Puget Sound, Washington. Also included in the study were samples of Coenobita perlatus (land hermit crab) and Lambis lambis (snail) which were captured in Eniwetok Atoll in Micronesia. These organisms

represented six invertebrate phyla. A total of 220 pure cultures of bacteria were isolated and identified. It was observed that, with the exception of Lambis lambis and Pseudostylochus ostreophagus, which are littoral zone animals, the distribution of bacterial genera within the samples taken from animals belonging to widely different taxonomic groups was similar. The predominant bacterial genus present, in all cases except the two mentioned above, was Pseudomonas. Flavobacterium and Achromobacter species were also present in significantly large proportions. Lambis lambis yielded a bacterial flora consisting entirely of Pseudomonas and Achromobacter. Pseudostylochus ostreophagus, on the other hand, yielded Bacillus, Aerobacter and Alcaligenes species. The authors noted that 89% of the Pseudomonas strains isolated from L. opalescens produced a green fluorescent pigment. It was also observed that fermentative types of bacteria occurred in significant numbers on C. gigas, H. panicea, L. lambis and P. ostreophagus, all of which are either sessile or associated with sessile animals. Oxidative bacteria were the major types isolated from the free-swimming L. opalescens and the drifting Aurelia species. The types of bacteria isolated from C. gigas by Colwell and Liston (1962a) were found to be similar to those found during an earlier study of the bacteriology of this animal (Colwell and Liston, 1960), in which the dominant genera were found to be Pseudomonas, Vibrio and Flavobacterium.

Beeson and Johnson (1967) studied the natural bacterial flora of the bean clam, Donax gouldi. Forty-seven bacterial strains were isolated and identified. The predominant genus was Vibrio, which comprised 50% of the isolates, followed by Pseudomonas (17%). Three strains were identified as Escherichia Castellani and Chalmers 1919 species and the remaining strains were left unassigned.

In view of the paucity of knowledge of the bacterial flora of coelenterates, Doores and Cook (1976) characterized the aerobic, mesophilic, heterotrophic bacteria found on the medusae of the sea nettle, Chrysaora quinquecirrha, in Chesapeake Bay. Computer analysis of 208 unknown bacterial strains isolated from the sea nettle permitted the identification of 15 groups of bacteria, representing 133 of the strains. The remaining 75 strains failed to associate with any of the 15 groups at the 70% similarity level. Twelve of the groups (68.8% of the isolates) were identified as Vibrio, 2 groups (11.6% of the isolates) were identified as Pseudomonas, and 1 group (8.2% of the isolates) was identified as Bacillus. The authors noted that all of the bacilli were isolated from a group of moribund nettles and therefore reflected an abnormal condition. The remaining isolates were distributed as follows: Flavobacterium (2.4%), Acinetobacter (2.4%), Moraxella (1.9%), Cytophaga (1.9%), and gram-positive cocci (1.4%).

Simidu et al. (1971) studied the bacterial flora of phytoplankton and zooplankton in Nishiura Bay, Japan.

These authors found that Vibrio and Aeromonas together comprised more than 70% of the bacterial strains isolated from the plankton samples. Pseudomonas comprised approximately 15% of the strains.

One of the few attempts to characterize the natural bacterial flora of echinoderms is that of Unkles (1977) who studied the sea urchin, Echinus esculentus. Three anatomical sites were sampled for their bacterial flora: the gut, the coelomic fluid, and the peristomial membrane. Approximately 200 specimens of E. esculentus were examined, the animals having been collected over an 18-month period at approximately monthly intervals. A total of 85 bacterial isolates from the sea urchins and, for comparison, 26 strains from sand and seawater were identified. Overall, the main genera were Pseudomonas and Vibrio. From seawater and sand, and from peristomial membrane there was a high percentage (approximately 25%) of gram-positive bacteria, but none was found in the gut or coelomic fluid. From the latter sites the predominant genera were Vibrio, followed by Pseudomonas, Aeromonas, and Flavobacterium, in that order. A few isolates of Moraxella and Acinetobacter (formerly classified as Achromobacter) were made from the peristomial membrane, and from sand and seawater.

Sochard et al. (1979) studied bacteria associated with the surface and gut of five species of marine copepod. A total of 329 strains of bacteria were isolated. Fifty-five percent of these strains were identified as belonging

to the genus Vibrio and 22% were identified as Pseudomonas species. Three percent of the isolates were identified as Cytophaga or Flavobacterium, 17% were unidentified, and 2 isolates were assigned to the genus Chromobacterium.

Boyle and Mitchell (1981) studied the bacterial flora of the marine wood-boring isopod Limnoria lignorum. Sixty-six strains of bacteria were isolated and the predominant genera were found to be Aeromonas, Pseudomonas, Vibrio and Acinetobacter.

Atlas et al. (1982) characterized 130 bacterial strains isolated from the Arctic amphipod Boeckosimus affinis and found the dominant bacterial flora to consist of gram-negative, motile, facultatively anaerobic rods which appeared to be members of the genus Vibrio.

The bacterial flora of marine sponges has been investigated by several people, including Madri et al. (1971), Reiswig (1975), Imhoff and Trüper (1976), and Wilkinson (1978a, 1978b, 1978c).

Madri et al. (1971) studied the bacterial flora of the redbeard sponge, Microcionia prolifera, and found that it consisted of species of Pseudomonas, Achromobacter, Flavobacterium, Corynebacterium (Lehmann and Neumann 1896), Micrococcus, Vibrio, and Aeromonas. They also found gram-negative, non-fermentative rods which could not be identified. Madri et al. (1971) also demonstrated quantitative differences in certain genera from month to month over a 6-month period. Aeromonas and Vibrio were isolated

rarely and irregularly. Pseudomonas predominated during the summer months with a corresponding decrease in the level of coryneforms, while Achromobacter and Flavobacterium levels remained relatively constant.

In this study of scallops the predominant flora of Na^+-requiring bacteria was Alteromonas and Vibrio. There were two kinds of Alteromonas, one being A. haloplanktis and another that closely resembled A. haloplanktis but was capable of aromatic ring cleavage and had a slightly higher moles % G + C in its DNA. These differences are sufficient to name the latter group of strains A. haloplanktis biotype II, although further study may show that there is sufficient grounds for the creation of a new species. For now, it is proposed that strains of A. haloplanktis not capable of aromatic ring cleavage be called biotype I and strains capable of ortho cleavage be called biotype II.

As shown from the formation of clusters by cluster analysis there were three distinct groups of Vibrio associated with the scallops. The feature by which these groups could be distinguished was the range of organic compounds which they utilized as sole sources of carbon and energy. The strains in cluster B utilized relatively few carbon compounds. The vibrios in cluster E used a wider range of compounds and those in cluster F used the most.

In the original study of the scallops (Powell, 1978) it was shown that 85% of the strains required Na^+ for growth. A further 11% of the strains were identified as

Pseudomonas fluorescens Migula 1895 and these did not require Na^+ for growth. Therefore, the genera associated with the scallops are Pseudomonas, Alteromonas and Vibrio. The latter two groups have a requirement for Na^+ in the growth medium. This is one of the first studies to report strains of Alteromonas species associated with marine invertebrates. However, because the genus Alteromonas was not officially recognized until listed in the Approved Lists of Bacterial Names published by the I.C.S.B. (Ad Hoc Committee, 1980), members of the genus would have been reported as Pseudomonas in earlier studies.

It is of interest to note that the marine pseudomonad B-16, which has been the object of extensive physiological studies by MacLeod and coworkers (reviewed by MacLeod 1965, 1968), has been identified as Alteromonas haloplanktis strain 214 (Baumann et al., 1972; Reichelt and Baumann, 1973b). This strain was isolated from a marine clam captured in the vicinity of Vancouver Island. Therefore, it is apparent that A. haloplanktis is indigenous to both the western Atlantic and eastern Pacific oceans.

Considering that marine algae elaborate and excrete large numbers and amounts of organic compounds, which are incorporated as structural components of the outer mucilage of the algae (O'Colla, 1962), and that heterotrophic bacteria commonly exhibit chemotaxis to concentration gradients of organic compounds (Bell and Mitchell, 1972), the surfaces of large, multicellular

marine algae should provide extremely favourable substrates for the growth of epiphytic, heterotrophic bacteria (Sieburth et al., 1974). Despite this, however, there are only a few comprehensive reports about the bacterial flora of macroscopic marine algae (Chan and McManus, 1969; Laycock, 1974; Kong and Chan, 1979; Shiba and Taga, 1980).

Chan and McManus (1969) studied the bacterial populations of two associated littoral marine algae, Polysiphonia lanosa and Ascophyllum nodosum, and the environmental seawater. Twenty-five strains of bacteria that occurred frequently were identified to genus. The most frequently occurring strains belonged to the genera Vibrio and Flavobacter. Eight of each genus were present. Three strains belonged to the genus Escherichia, two were pseudomonads, and one each belonged to the genera Sarcina Goodsir, 1842, Staphylococcus Rosenback, 1884, and Achromobacter. One isolate was Rhodotorula Harrison, 1928, a pink yeast.

Over a thirteen-month period, Laycock (1974) investigated the bacteria associated with the surface of fronds of a sublittoral brown alga, Laminaria longicruris. A psychrophilic population was associated with the alga during the winter and a mesophilic population with the decaying frond during the summer. Bacteria which hydrolyze laminaran were characteristic of the psychrophilic flora and a group of bacteria hydrolyzing mannitol, protein and alginate were characteristic of the mesophilic flora.

Increases in the numbers and proportions of bacteria utilizing plant substrates accompanied frond decomposition. Forty-two hundred bacterial cultures were isolated and identified to the genus level. The most common genera isolated were Vibrio and Pseudomonas groups 1 and 2.

Kong and Chan (1979) isolated a total of fifty-eight strains of heterotrophic bacteria from various marine algae. These were the green algae Ulva lactuca, Chaetomorpha brachygona and Codium cylindricum, the brown algae Sargassum hemiphyllum and Ectocarpus siliculosus, and the red algae Polysiphonia lanosa and Hypnea charoides. The bacterial strains belonged to seven genera, including Pseudomonas, Vibrio, Alteromonas, Xanthomonas Dowson, 1939, Achromobacter, Flavobacterium and Micrococcus. Strains of Micrococcus and Achromobacter were the dominant flora associated with the green algae. The yellow and orange-pigmented strains of Flavobacterium and Xanthomonas, and strains of Alteromonas comprised the major flora of the brown algae. The dominant bacterial flora of the red algae was intermediate between that associated with the green and brown algae. However, strains representing all of the assigned bacterial genera were present.

Kong and Chan (1979) found that a large proportion of the bacterial strains isolated from the marine algae produced extracellular enzymes such as gelatinase, amylase and alginase which allowed the bacteria to utilize various algal substrates.

Shiba and Taga (1980) studied the bacterial populations associated with four species of seaweed and the environmental seawater in Nabeta Inlet and Otsuchi Bay, Japan. The seaweed species studied were the green algae Monostroma nitidum and Enteromorpha linza, the red alga Porphyra suborbiculata, and the brown alga Eisenia bicyclis. The bacterial populations of the green and red algae were generally characterized by a predominance of the pigmented genera Flavobacterium and Cytophaga. In contrast, the bacterial flora of the brown alga and seawater samples were characterized by a predominance of the genera Acinetobacter and Moraxella. Small numbers of pseudomonads and vibrios were isolated from all samples studied.

In this study of the seaweed Alaria esculenta, marine vibrios and some pseudomonads (namely P. marina) made up the majority of the bacteria associated with the fronds. Ten percent of the strains described earlier by Hollohan (1980) did not require Na^+ for growth and were not studied here. It was observed in the present study that the vibrios that were isolated from fresh fronds of A. esculenta were potent producers of exoenzymes. Chan and McManus (1969), Laycock (1974) and Kong and Chan (1979) all observed that bacteria associated with marine algae elaborated a number of exoenzymes specific for complex polymers present in the algal tissue.

The only study other than the one reported here which has characterized the bacterial flora of a marine

alga over an extended period of time is that of Laycock (1974) who studied fronds of Laminaria longicruris over a thirteen-month period. However, since Laycock (1974) identified the bacterial strains only at the genus level and studied only fresh seaweed blades, it is difficult to draw parallels between that study and the one reported here. Laycock (1974) found that the dominant genera present over the study period were Vibrio and Pseudomonas. However, there is evidence that there was a succession of bacterial species during the 13-month period since the bacterial population associated with the fronds during the summer were found to be mesophilic, whereas the bacteria associated with the fronds during the winter were found to be psychrophilic. It is possible, though, that these bacteria were the same species but became adapted to the ambient temperatures present throughout the long study period.

Laycock (1974) also found that numbers of bacteria capable of producing an extracellular alginase and gelatinase remained consistently high throughout the year, while bacteria capable of producing an extracellular laminaranase were isolated only from October to April.

Kong and Chan (1979), who studied the bacterial flora of seven species of macroscopic marine algae which were collected on a single occasion, found that a high proportion of the strains isolated were capable of producing an extracellular gelatinase, alginase and cellulase.

Since neither Laycock (1974) nor Kong and Chan (1979) studied decomposed marine algae, it is impossible to say whether the low proportion of bacterial strains capable of producing various exoenzymes on decomposed A. esculenta blades observed in this study is typical of marine algae in general or a phenomenon that is possibly unique to A. esculenta. The former hypothesis is most likely to be the case since it has been shown (Haug and Jensen, 1954; Percival and McDowell, 1967) that alginate, gelatin, cellulose and laminaran comprise a great proportion of the organic matter in fresh fronds of seaweeds such as A. esculenta and several Laminaria species. Therefore, it would be expected that the greatest proportion of bacteria capable of producing these various exoenzymes would be isolated from samples of fresh algae. Likewise, samples of decomposed algae would presumably yield smaller numbers of bacteria capable of producing the same exoenzymes since the amount of these substrates in the decomposed algal tissue is no doubt much lower than that in the fresh living algal tissue.

Identification schemes derived from that of Shewan et al. (1960) have been used in the study of planktonic marine bacteria by several investigators, including Pfister and Burkholder (1965), Kaneko and Colwell (1973), Gauthier et al. (1975), Simidu et al. (1977), Austin et al. (1979), Kaneko et al. (1979), and Hauxhurst et al. (1980).

Austin et al. (1979) conducted a comparative study of the bacterial flora of the water of Chesapeake Bay and Tokyo Bay. A total of one hundred and ninety-five aerobic, heterotrophic bacterial strains isolated from the two bays were examined for one hundred and fifteen biochemical, cultural, morphological, nutritional, and physiological characters. Numerical analysis of the data revealed that 77% of the isolates could be grouped into 30 phena and presumptively identified as Acinetobacter - Moraxella, Caulobacter Henrici and Johnson 1935, coryneforms, Pseudomonas, and Vibrio species. Vibrio and Acinetobacter species were found to be common in the estuarine waters of Chesapeake Bay, whereas Acinetobacter - Moraxella and Caulobacter predominated in Tokyo Bay waters, at the sites sampled in the study.

Kaneko et al. (1979) studied the taxonomy of five hundred and fifty-three bacterial strains isolated from the Arctic Beaufort Sea. The strains were tested for three hundred characters. Computer analysis of the results distinguished 27 phenotypic clusters. Fourteen clusters consisted of bacteria which had been isolated at 4°C. The remaining thirteen clusters were bacteria which had been isolated at 20°C. The predominant genus identified was Flavobacterium (9 clusters), followed by Vibrio and Pseudomonas. Seven of the clusters could not be identified. All of the isolates were psychrophilic or psychrotrophic.

The results of Kaneko et al. (1979) contrasted with those of Pfister and Burkholder (1965) who found Pseudomonas species to be dominant and pigmented bacteria to comprise a low proportion of the bacterial population in Antarctic waters.

Hauxhurst et al. (1980) characterized two hundred and forty-seven bacterial strains isolated from the northwest Gulf of Alaska in October 1975 and one thousand and ten bacterial strains isolated from the northeast Gulf of Alaska in March 1976. Computer analysis of the results revealed 24 clusters from the northeast Gulf isolates and 12 clusters from the northwest Gulf isolates. The dominant organisms in the northeast Gulf of Alaska were identified as belonging to the Acinetobacter - Moraxella group; these organisms were not found in the northwest Gulf of Alaska. Several clusters from samples collected from both the northeast and northwest Gulf regions were tentatively identified as belonging to the Vibrio - Aeromonas group. A high proportion of the strains from both regions were pigmented; several clusters of Flavobacterium were identified. There was a notable lack of Pseudomonas species in both regions of the Gulf of Alaska.

Overall, the results of the former studies tend to indicate that there is no obvious relationship between groups of bacteria identified and the source of the strains. The distribution of bacterial genera within samples taken from animals and plants belonging to widely different taxonomic groups is similar. The same is true for water samples from

geographically distant areas. In many cases a wide range of bacterial genera have been identified. The most common genera found were Vibrio, Pseudomonas and Flavobacterium. Such diverse genera as Cytophaga, Escherichia, Bacillus, Acinetobacter, Micrococcus, Aeromonas, Chromobacterium and Corynebacterium have also been found in various habitats. However, it must be remembered that for the most part these researchers identified the bacterial isolates only at the genus level and many used simple taxonomic schemes. The earlier studies were conducted before the Na^+ requirement was popularly recognized as a distinguishing characteristic of true marine bacteria. Consequently, many of these studies have included various types of bacteria, many of which could have been contaminants from terrestrial sources (eg. Micrococcus, Bacillus and Escherichia).

In the study reported here a comprehensive taxonomic scheme was used. Strains were identified, when possible, at the species level. Although the study was restricted to bacteria that required Na^+ for growth, 85 to 90% of the strains isolated from the scallops and seaweeds had this characteristic (Powell 1978; Hollohan, 1980) and therefore the majority of the strains originally isolated from the two sources were represented in this study.

The results show that 86% of the cold ocean isolates fell within six well-defined clusters. The bacteria in these clusters could be identified as belonging

to a small number of genera, namely Vibrio, Pseudomonas and Alteromonas. This is in contrast to the great number of genera found in some of the studies mentioned earlier. It has been determined that the six clusters may represent at least eight species of bacteria. This small number of species would suggest that the waters of the northwest Atlantic near Newfoundland may have only a few species of marine bacteria relative to the total number known. Approximately forty species of marine bacteria that require Na^+ for growth have been described (Baumann and Baumann, 1981). However, different groups of bacteria were associated with the scallop and seaweed and therefore it is possible that if more habitats are studied more species of marine bacteria will be found. This would be a subject suitable for further study.

The groups of bacteria associated with P. magellanicus were different than those found on A. esculenta. Bacteria from marine invertebrates and seaweeds have not been compared before in a single study. Colwell and Liston (1960, 1962a), Simidu et al. (1971) and Sochard et al. (1979) studied bacteria from various species of invertebrates and concluded that specific bacterial genera were not associated with specific sources. However, the methods used to differentiate the strains may not have been sufficiently detailed to detect differences beyond those that distinguish genera. The same problem has existed in

the study of bacteria isolated from seaweed (Chan and McManus, 1969; Laycock, 1974; Kong and Chan, 1979; Shiba and Taga, 1980).

The observation that the bacterial flora of the scallop was different from that of the seaweed does not necessarily mean that these differences are constant. The scallops were collected at a different site than were the seaweed samples, and the times of collection were approximately two years apart. Had the scallops and the seaweed been collected at the same time and at the same site it would provide evidence that the bacterial flora was different because of the association of the flora with the host. The differences observed in this study may have been due to population fluctuations with time or to geographical location. The answer to this question would be important to environmental biologists. It is possible that sampling at one or two times or one or two locations may not give a true picture of the typical bacterial flora. An answer to this question was beyond the scope of this thesis.

One of the fundamental purposes of detailed taxonomic studies is to provide simplified identification schemes or diagnostic tables. Approximately fifty important characters for differentiating species of marine bacteria in this region have been presented in this thesis. These tests could be used to study changes in the population of the bacterial flora both according to habitat and time.

SUMMARY OF CONCLUSIONS

Cold ocean marine eubacteria from the Northwest Atlantic near Newfoundland were characterized:

1. The predominant bacteria were from the genera *Pseudomonas*, *Alteromonas* and *Vibrio*.
2. The pseudomonads were identified as *P. marina*.
3. The alteromonads were identified as *A. haloplanktis* and some may be a new biotype.
4. Clusters of psychrophilic vibrios were obtained. These were not identified to the species level but each cluster may represent a new species of marine vibrio.
5. Different groups of bacteria were associated with the different habitats. The scallops had a different population of bacteria than did the seaweed. Furthermore, there was a succession of vibrios that populated the seaweed during the biodegradation process.

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APPENDIX A

Table 1: LIST OF OTU USED IN THE NUMERICAL ANALYSIS.

Number assigned in this study	Source
	Fermentative strains isolated from seaweed (Hollohan, 1980):
1	D1-1
2	D1-2
3	D1-3
4	D1-4
5	D1-5
6	D1-6
7	D1-7
8	D1-8
9	D1-9
10	D1-10
11	D1-11
12	D1-14
13	D1-15
14	D1-16
15	D1-18
16	D1-19
17	D1-22
18	D1-23
19	D1-26
20	D1-28
21	D1-30

Table 1 continued ...

Number assigned in this study	Source
22	D1-32
23	D1-39
24	D1-40
25	D2-4
26	D2-8
27	D2-9
28	D2-10
29	D2-11
30	D2-12
31	D2-14
32	D2-17
33	D2-18
34	D2-19
35	D2-23
36	D2-25
37	D2-27
38	D2-30
39	D2-33
40	D2-35
41	D2-37
42	D2-38
43	D2-39
44	D2-40

Table 1 continued ...

Number assigned in this study	Source
45	D2-42
46	D2-43
47	D2-44
48	D3-4
49	D3-6
50	D3-7
51	D3-8
52	D3-9
53	D3-11
54	D3-12
55	D3-13
56	D3-14
57	D3-15
58	D3-16
59	D3-17
60	D3-18
61	D3-24
62	D3-25
63	D3-28
64	D3-29
65	D3-30

Table 1 continued ...

Number assigned in this study	Source
	Oxidative strains isolated from seaweed (Hollohan, 1980):
66	D1-12
67	D1-13
68	D2-2
69	D2-3
70	D2-5
71	D2-6
72	D2-16
73	D2-21
74	D2-22
75	D2-29
76	D2-31
77	D2-32
78	D2-41
79	D2-45
80	D3-19
81	D3-21
82	D3-22
83	D3-26
84	D3-27

Table 1 continued ...

Number assigned in this study	Source
	Fermentative strains isolated from scallop (Powell, 1978):
85	2
86	10
87	12
88	14
89	17
90	20
91	26
92	33
93	34
94	36
95	40
96	49
97	50
98	53
99	57
100	62
101	66
102	67
103	68
104	69
105	70

Table 1 continued ...

Number assigned in this study	Source
106	72
107	73
108	75
109	77
110	78
111	79
112	81
113	89
114	92
115	94
116	95
	Oxidative strains isolated from scallop (Powell, 1978):
117	1
118	3
119	5
120	6
121	13
122	16
123	22
124	28
125	29
126	31

Table 1 continued ...

Number assigned in this study	Source
127	37
128	38
129	39
130	41
131	42
132	43
133	44
134	46
135	47
136	51
137	52
138	55
139	58
140	65
	Strains obtained from the American Type Culture Collection (ATCC), Rockville, Maryland:
141	ATCC 29660
142	ATCC 29659
143	ATCC 27562
144	ATCC 25914
145	ATCC 27043
146	ATCC 27126

Table 1 continued ...

Number assigned in this study	Source
147	ATCC 25915
148	ATCC 27125
149	ATCC 27132

Table 2: Number assigned to tests used in the numerical analysis.

Number	Test
1	PHB accumulation
2	oxidase
3	catalase
4	arginine dihydrolase
5	denitrification
6	$NO_3 \rightarrow NO_2$
7	growth at 4°C
8	growth at 35°C
9	growth at 40°C
10	luminescence
11	laminaranase
12	amylase
13	agar digestion
14	gelatinase
15	lipase
16	alginase
17	chitinase
18	cellulase (CMC)
19	D-ribose
20	D-xylose
21	D-arabinose
22	L-arabinose
23	sucrose

Table 2 continued ...

Number	Test
24	trehalose
25	maltose
26	cellobiose
27	melibiose
28	lactose
29	L-rhamnose
30	D-glucose
31	D-mannose
32	D-galactose
33	D-fructose
34	saccharate
35	mucate
36	D-galacturonate
37	glycine
38	L-α-alanine
39	D-α-alanine
40	β-alanine
41	L-serine
42	L-leucine
43	L-isoleucine
44	DL-norleucine
45	L-valine
46	L-aspartate
47	L-lysine

Table 2 continued ...

Number	Test
48	DL-citrulline
49	DL-α-aminobutyrate
50	γ-aminobutyrate
51	DL-α-aminovalerate
52	DL-arginine
53	DL-ornithine
54	N-acetylglucosamine
55	succinate
56	fumarate
57	DL-lactate
58	pyruvate
59	glycerol
60	L-proline
61	salicin
62	isobutyrate
63	isovalerate
64	malonate
65	DL-β-hydroxybutyrate
66	sorbitol
67	inositol
68	benzoate
69	p-hydroxybenzoate
70	phenylacetate
71	quinate

Table 2 continued ...

Number	Test
72	δ-aminovalerate
73	betaine
74	sarcosine
75	hippurate
76	D-gluconate
77	D-glucoronate
78	valerate
79	DL-glycerate
80	α-ketoglutarate
81	propanol
82	L-threonine
83	L-histidine
84	putrescine
85	maleate
86	adipate
87	suberate
88	azelate
89	sebacate
90	L-phenylalanine
91	L-tryptophan
92	n-butanol
93	itaconate
94	allantoin
95	nicotinate

Table 2 continued ...

Number	Test
96	adonitol
97	glycolate
98	acetate
99	propionate
100	butyrate
101	heptanoate
102	pelargonate
103	ethanol
104	DL-malate
105	citrate
106	aconitate
107	mannitol
108	L-tyrosine
109	L-malate
110	fermentative breakdown of glucose
111	oxidative breakdown of glucose
112	aromatic ring cleavage

Table 3. Data matrix for the 149 OTU and 112 characters.

OTU #	TEST #																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	+	-	+
2	-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	+	-	+	-	+
3	+	+	-	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	-	+
4	+	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	-	+	-	+
5	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	-	+
6	+	+	+	-	-	-	+	-	-	-	+	+	-	+	+	-	-	+	-	+
7	+	+	-	-	-	-	+	-	-	-	+	+	-	+	+	-	-	+	-	+
8	+	+	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	+	-	-
9	+	-	-	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	-	+
10	+	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
11	+	-	-	-	-	-	+	+	+	+	-	-	+	+	+	-	+	-	-	-
12	+	-	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-
13	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-
14	+	-	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-
15	-	+	+	-	-	-	+	+	+	-	+	+	-	+	+	+	-	-	+	+
16	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	-	+	-	-
17	-	+	-	-	-	-	+	-	-	-	+	+	-	+	+	+	+	+	-	+
18	+	+	+	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
19	-	+	-	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	-	-
20	-	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	-	+	+	+
21	-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	-	-	+	-	-
22	-	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	-	+	-	-
23	-	-	-	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	-	+
24	-	-	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	-	+
25	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+
26	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	+	-
27	-	+	-	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-
28	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
29	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	-
30	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	-	+	-
31	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	+	-
32	-	+	-	-	+	+	+	-	-	-	+	+	-	+	+	+	-	-	+	-
33	-	+	-	+	+	+	+	-	-	-	+	+	-	+	+	+	-	-	+	-
34	-	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
35	+	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+	-	-	+	-
36	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-
37	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-
38	+	+	+	-	-	-	+	+	+	-	+	+	-	+	-	+	-	-	-	-
39	-	+	-	-	+	+	+	-	-	-	+	-	+	+	+	+	-	-	+	-
40	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-

Table 3 continued ...

DTU #	TEST #																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	-	-	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-	+	-	-
2	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-	-
3	-	-	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-	+	-	-
4	-	-	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+
5	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	+	+
6	-	-	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-	+	-	-
7	-	-	+	+	+	-	+	+	-	+	-	+	-	-	-	-	-	+	-	-
8	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-	-
9	-	-	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-	+	-	-
10	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	+	+
11	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-	-
12	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	+	-
13	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-	-
14	-	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	-	+	+	-
15	-	-	+	-	+	+	+	+	-	+	-	+	+	-	-	-	-	+	+	+
16	-	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	-	+	+	+
17	-	-	+	-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	-
18	-	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	-	+	+	+
19	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	+	-	-
21	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-	-
22	-	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	-	-
23	-	-	+	-	+	+	+	+	-	+	-	+	-	-	-	-	-	+	-	-
24	-	-	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-
25	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	-	+	-
26	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
27	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	+	-	+	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
30	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
31	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+
32	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	-
33	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
34	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
35	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
36	+	+	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-
37	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
38	-	-	-	+	-	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+
39	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
40	-	-	-	+	+	+	-	+	-	+	+	+	+	-	-	-	-	-	+	-

Table 3 continued ...

OTU #	TEST #																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
1	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
2	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	-
3	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
4	-	-	+	-	-	-	-	+	-	+	-	+	-	-	+	+	-	+	+	+
5	-	+	+	+	-	+	-	+	-	+	-	+	+	-	+	+	-	+	+	+
6	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
7	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
8	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
9	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
10	-	+	+	+	-	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+
11	-	-	+	-	-	-	-	+	-	+	-	+	-	-	+	+	-	+	+	+
12	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
13	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
14	-	-	+	-	-	+	-	+	-	-	-	+	-	-	+	+	-	+	+	+
15	+	+	+	+	-	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+
16	+	+	+	-	-	-	-	+	-	+	-	+	-	-	+	+	-	+	+	+
17	-	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	-
18	+	+	+	+	-	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+
19	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-
20	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	+	+	-	+
21	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	-
22	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
23	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	-	+
24	-	+	+	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+	-	+
25	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+
26	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
27	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
28	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
29	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
30	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
31	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+
32	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
33	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
34	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
35	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
36	+	-	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+
37	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	-	-	+	+
38	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
39	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
40	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+

Table 3 continued ...

DTU #	TEST #																			
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
2	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	-	-	-
3	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
4	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	-
5	-	-	-	-	-	-	+	-	+	-	-	-	+	+	-	+	+	-	+	-
6	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
7	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
9	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
10	-	-	+	+	+	-	+	-	+	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	+	-	-	-	-
12	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-
13	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-
14	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
15	-	-	+	+	+	-	+	-	-	-	-	+	+	+	-	+	-	+	+	+
16	-	-	-	-	-	-	+	-	+	-	-	-	+	+	-	+	-	+	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
18	-	-	+	+	+	-	+	-	+	-	-	-	+	+	-	+	-	+	+	+
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-
21	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
24	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+
25	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-	+
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
31	+	-	+	-	+	+	+	-	-	-	+	-	+	+	-	+	+	+	+	+
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
36	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-
37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
38	+	-	+	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	+	+
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+

Table 3 continued ...

OTU #	TEST H																			
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
15	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
18	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
23	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
25	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
26	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
27	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
28	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
30	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
31	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
32	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
33	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
34	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
35	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
36	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
37	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
38	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
39	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
40	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-

Table 3 continued ...

OTU #	TEST #											
	101	102	103	104	105	106	107	108	109	110	111	112
1	-	+	-	-	+	-	+	+	+	+	-	-
2	-	+	-	-	+	-	+	+	+	+	-	-
3	-	+	-	-	+	-	+	+	+	+	-	-
4	-	+	-	-	+	-	+	+	+	+	-	-
5	-	+	-	-	+	-	+	+	+	+	-	-
6	-	+	-	-	+	-	+	+	+	+	-	-
7	-	+	-	-	+	-	+	+	+	+	-	-
8	-	-	-	-	-	-	+	+	+	+	-	-
9	-	+	-	-	+	-	+	+	+	+	-	-
10	+	+	+	+	+	+	+	+	+	+	-	-
11	-	+	-	-	+	-	+	+	+	+	-	-
12	-	+	-	+	-	-	+	+	+	+	-	-
13	-	-	-	-	-	-	+	+	+	+	-	-
14	-	-	-	-	+	-	+	+	+	+	-	-
15	+	+	+	+	+	+	+	+	+	+	-	-
16	-	-	-	-	-	-	+	+	+	+	-	-
17	-	+	-	-	+	-	+	+	+	+	-	-
18	+	+	+	+	+	+	+	+	+	+	-	-
19	-	-	-	-	+	-	-	-	+	+	-	-
20	-	+	-	-	+	+	+	+	+	+	-	-
21	-	+	-	-	+	-	+	+	+	+	-	-
22	-	+	-	-	+	-	+	+	+	+	-	-
23	-	+	-	-	+	+	+	+	+	+	-	-
24	-	-	+	+	+	+	+	+	+	+	-	-
25	-	-	-	+	+	+	+	+	+	+	-	-
26	-	-	-	+	+	+	+	-	+	+	-	-
27	-	-	-	+	+	+	+	-	+	+	-	-
28	-	-	-	+	+	+	-	-	-	+	-	-
29	-	-	-	+	+	+	+	-	+	+	-	-
30	-	-	-	+	+	+	+	-	+	+	-	-
31	-	-	+	+	+	+	+	+	+	+	-	-
32	-	-	-	+	+	+	+	-	+	+	-	-
33	-	-	-	+	+	+	+	-	+	+	-	-
34	-	-	-	+	+	+	+	-	+	+	-	-
35	-	-	-	+	+	+	+	-	+	+	-	-
36	-	-	-	+	+	+	+	-	+	+	-	-
37	-	-	-	+	+	+	+	-	+	+	-	-
38	+	+	+	+	+	+	+	+	+	+	-	-
39	-	-	-	+	+	+	+	-	+	+	-	-
40	-	-	-	+	+	+	+	-	+	+	-	-

Table 3 continued ...

OTU #	TEST #																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
41	+	+	+	-	+	+	+	-	-	-	+	+	-	+	+	+	-	-	+	-
42	-	+	+	+	+	+	+	-	-	-	+	-	-	+	+	+	-	-	+	-
43	-	+	+	+	+	+	+	-	-	-	+	-	-	+	+	+	-	-	+	-
44	-	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	-	-	+	-
45	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-
46	-	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+	-	-	+	-
47	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-
48	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
49	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	+	-
50	+	+	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-
51	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	+	-
52	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
53	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
54	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
55	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-
56	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
57	+	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
58	-	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
59	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
60	+	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
61	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-
62	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
63	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	-	-
64	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	-	+	-
65	-	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-
66	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
67	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
68	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	+	-
69	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	+	-
70	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	+	-
71	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-
72	-	+	+	-	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
73	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	-
74	+	+	+	-	+	+	+	+	+	-	-	+	-	+	+	+	-	-	+	-
75	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	-
76	+	+	+	-	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	-
77	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-
78	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-
79	+	+	+	-	+	+	+	+	+	-	-	+	-	+	-	+	-	-	+	-
80	+	+	+	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-

Table 3 continued ...

OTU #	TEST #																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
41	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
42	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-
43	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
44	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
45	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
46	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
47	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
48	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
49	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
50	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
51	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
52	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
53	-	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
54	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
55	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
56	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
57	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
58	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
59	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
60	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
61	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
62	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
63	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
65	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-
66	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
67	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-
68	-	-	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	+	+	+
69	-	-	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	+	+	+
70	-	-	-	-	-	+	-	-	-	+	+	+	+	-	-	-	-	+	+	+
71	-	-	-	-	-	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+
72	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	-
73	-	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	+	+	+	+
74	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+
75	-	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	+	+	+	+
76	-	-	+	+	-	+	-	-	-	+	+	+	-	-	-	-	+	+	+	-
77	-	-	-	+	-	+	-	-	-	+	-	+	+	-	-	-	+	+	+	+
78	-	-	-	+	-	+	-	-	-	+	-	+	+	-	-	-	+	+	+	+
79	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+
80	-	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	+	+	+	+

Table 3 continued . . .

DTU #	TEST #																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
41	+	-	-	-	-	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+
42	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+
43	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
44	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
45	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
46	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
47	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
48	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
49	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
50	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
51	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
52	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
53	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
54	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
55	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
56	+	-	-	-	-	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+
57	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
58	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
59	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
60	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
61	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
62	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+
63	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	+	-	-
64	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
65	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+
66	-	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	-	+
67	-	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	-	+
68	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
69	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
70	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
71	+	+	+	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
72	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
73	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+
74	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
75	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+
76	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+
77	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
78	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
79	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
80	+	+	-	-	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+

Table 3 continued ...

OTU #	TEST #																			
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
58	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
66	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
67	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
68	-	-	+	+	+	-	+	-	-	-	-	-	+	+	-	+	-	+	+	+
69	-	-	+	+	+	-	+	-	-	-	-	-	+	+	-	+	-	+	+	+
70	-	-	+	+	+	-	+	-	-	-	-	-	+	+	-	+	-	+	+	+
71	-	-	+	+	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+
72	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+
73	-	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+	+	+	+
74	-	-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+
75	-	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	-	+	+	+
76	-	+	+	+	+	-	-	-	-	-	+	+	-	-	-	+	-	+	-	+
77	-	-	+	+	+	-	+	-	-	+	+	-	+	+	-	+	-	+	+	+
78	-	-	+	+	+	-	+	-	-	+	+	-	+	+	-	+	-	+	+	+
79	-	-	+	+	+	-	+	-	-	+	+	-	+	+	-	+	+	+	+	+
80	-	+	+	+	+	-	+	-	-	+	+	+	+	+	-	+	-	+	+	+

Table 3 continued ...

OTU #	TEST #																			
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
41	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
42	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
43	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
44	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
45	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
46	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
47	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
48	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
49	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
50	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
51	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
52	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
53	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
54	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
55	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
56	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
57	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
58	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
59	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
60	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
61	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
62	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
64	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
65	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
66	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
67	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
68	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
69	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
70	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
71	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
72	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
73	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+	-	+	+	+	+
74	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
75	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+	-	+	+	+	+
76	-	+	-	+	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+
77	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
78	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
79	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
80	+	+	-	+	+	+	-	-	-	+	-	+	-	+	-	-	+	+	+	+

Table 3 continued ...

OTU #	TEST #											
	101	102	103	104	105	106	107	108	109	110	111	112
41	-	-	-	+	+	+	+	-	+	+	-	-
42	-	-	-	+	+	+	+	-	+	+	-	-
43	-	-	-	+	+	+	+	-	+	+	-	-
44	-	-	-	+	+	+	+	-	+	+	-	-
45	-	-	-	+	+	+	+	-	+	+	-	-
46	-	-	-	+	+	+	+	-	+	+	-	-
47	-	-	-	+	+	+	+	-	+	+	-	-
48	-	-	-	+	+	+	+	-	+	+	-	-
49	-	-	-	+	+	+	+	-	+	+	-	-
50	-	-	-	+	+	+	+	-	+	+	-	-
51	-	-	-	+	+	+	+	-	+	+	-	-
52	-	-	-	+	+	+	+	-	+	+	-	-
53	-	-	-	+	+	+	+	-	+	+	-	-
54	-	-	-	+	+	+	+	-	+	+	-	-
55	-	-	-	+	+	+	+	-	+	+	-	-
56	-	-	-	+	+	+	+	-	+	+	-	-
57	-	-	-	+	+	+	+	-	+	+	-	-
58	-	-	-	+	+	+	+	-	+	+	-	-
59	-	-	-	+	+	+	+	-	+	+	-	-
60	-	-	-	+	+	+	+	-	+	+	-	-
61	-	-	-	-	+	+	+	-	+	+	-	-
62	-	-	-	-	+	+	+	-	+	+	-	-
63	-	-	-	-	-	+	+	-	+	+	-	-
64	-	-	-	+	+	+	+	-	+	+	-	-
65	-	-	-	+	+	+	+	-	+	+	-	-
66	+	-	-	-	-	-	-	+	+	-	+	-
67	+	-	-	-	-	-	-	+	+	-	+	-
68	+	+	+	+	+	+	+	+	+	-	+	-
69	+	+	+	+	+	+	+	+	+	-	+	-
70	+	+	+	+	+	+	+	+	+	-	+	-
71	+	+	+	+	+	+	+	+	+	-	+	-
72	-	-	-	+	+	+	+	-	+	-	+	-
73	+	-	+	+	+	+	+	+	+	-	+	-
74	+	+	+	+	+	+	+	+	+	-	+	-
75	+	-	+	+	+	+	+	+	+	-	+	-
76	+	-	+	+	-	-	+	+	+	-	+	+
77	+	+	+	+	+	+	+	+	+	-	+	+
78	-	+	+	+	+	+	+	+	+	-	+	+
79	+	+	+	+	+	+	+	+	+	-	+	+
80	+	+	+	+	+	+	+	+	+	-	+	+

Table 3 continued ...

OTU #	TEST #																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
81	+	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	-	-	+	-
82	+	+	-	-	-	+	+	-	-	-	+	-	-	+	+	+	-	-	+	-
83	-	+	-	-	-	+	+	-	-	-	+	-	-	+	+	+	-	-	-	-
84	-	+	-	-	-	+	+	-	-	-	+	-	-	+	+	+	-	-	-	-
85	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
86	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-
87	-	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
88	+	+	-	-	-	+	+	-	-	-	-	-	-	+	+	-	+	-	-	-
89	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
90	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
91	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-
92	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
93	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
94	+	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	+	-	-	+
95	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
96	+	+	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
97	+	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
98	+	+	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
99	+	+	+	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
100	+	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
101	+	+	+	-	+	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-
102	-	+	+	-	+	+	+	-	-	-	+	+	-	+	+	-	-	-	-	-
103	-	+	+	-	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
104	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
105	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
106	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	+	-	-	+
107	-	+	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
108	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
109	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
110	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
111	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
112	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
113	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
114	-	-	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
115	-	+	+	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+	-
116	-	+	+	+	-	-	+	-	-	-	+	-	-	+	+	-	-	-	-	-
117	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-
118	-	+	+	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
119	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-
120	-	+	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-

Table 3 continued ...

OTU #	TEST #																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
81	-	-	+	+	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+
82	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+
83	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
84	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
85	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
86	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+
87	-	-	+	-	+	+	-	-	+	+	-	-	+	-	-	-	-	-	+	-
88	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
92	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
93	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
94	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
95	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-
96	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
97	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
98	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
99	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+
100	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
101	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-
102	-	+	+	+	+	-	-	-	-	+	-	-	+	-	-	+	+	+	+	-
103	-	-	-	-	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-
104	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	+	-
105	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	+	-
106	-	-	+	-	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-
107	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-
108	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	-
109	-	-	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-
110	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
111	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
112	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
113	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
114	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-
115	-	+	-	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	-
116	-	+	-	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+
117	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	-
118	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	+	-
119	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	+	-
120	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	-

Table 3 continued ...

OTU #	TEST #																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
81	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+
82	+	+	+	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+
83	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	+	-
84	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-
85	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-
86	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+
87	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
88	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-
89	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+	+	+	-	-
90	-	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	-
91	-	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
92	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
93	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	-
94	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
95	-	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
96	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
97	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
98	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	-
99	+	+	-	-	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
100	+	+	-	-	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+
101	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
102	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
103	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
104	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+
105	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+
106	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
107	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
108	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
109	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-
110	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-
111	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
112	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-
113	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-
114	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-
115	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
116	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
117	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
118	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
119	-	+	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	-	+	+
120	+	+	-	-	-	+	-	-	-	+	+	-	+	-	+	+	+	+	-	+

Table 3 continued ...

OTU #	TEST #																			
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
81	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+
82	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
86	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	+	-	+
87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
91	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	+
92	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	+
93	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+
94	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+
95	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	+
96	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+
97	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+
98	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+
99	-	+	-	-	+	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+
100	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+
101	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
102	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
103	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
104	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
105	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
106	-	+	+	+	+	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+
107	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
108	-	+	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+
109	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
110	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-
111	-	+	+	+	+	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
112	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+
113	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
114	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+
115	-	-	-	-	+	-	+	-	+	-	+	-	+	+	-	+	+	-	+	+
116	-	-	-	-	+	-	+	-	+	-	+	-	+	+	-	+	+	-	+	+
117	-	-	-	-	+	-	+	-	+	-	+	-	+	+	-	+	+	-	+	+
118	-	-	-	-	+	+	+	-	+	-	+	-	+	+	-	+	+	-	+	+
119	-	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+
120	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	-	-	+	+

Table 3 continued ...

OTU #	TEST #																			
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
81	+	-	-	+	+	+	-	-	-	+	-	+	-	+	+	-	+	+	+	+
82	+	-	-	+	+	+	-	-	-	+	-	+	-	+	+	-	+	+	+	+
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
86	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
87	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
91	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
92	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
94	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
95	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
96	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
99	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+
100	+	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	+	+	+	+
101	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+
102	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	+	+	+
103	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+
104	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+
105	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+
106	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+
107	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+
108	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
109	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
110	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-
111	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+
112	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+
113	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	+	+
114	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+
115	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	-
116	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	-	+
117	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	+	-	+	+	-
118	-	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	+	-
119	-	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	+	-
120	-	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	+	-

Table 3 continued...

OTU #	TEST #											
	101	102	103	104	105	106	107	108	109	110	111	112
81	+	+	+	+	+	+	+	+	+	-	+	+
82	+	+	+	+	+	+	+	+	+	-	+	+
83	-	-	-	-	-	-	-	-	-	-	+	-
84	-	-	-	-	-	-	-	-	-	-	+	-
85	-	-	-	+	-	-	-	-	+	+	-	-
86	-	-	-	+	-	+	+	+	-	+	-	-
87	-	-	-	+	-	-	+	+	+	+	-	-
88	-	-	-	+	-	-	+	+	+	+	-	-
89	-	-	-	-	-	-	-	+	-	+	-	-
90	-	-	-	+	-	-	-	-	+	+	-	-
91	-	-	-	+	-	-	-	+	+	+	-	-
92	-	-	-	+	-	-	-	+	+	+	-	-
93	-	-	-	+	-	-	+	+	+	+	-	-
94	-	-	-	+	-	-	+	+	+	+	-	-
95	-	-	-	+	-	-	-	+	+	+	-	-
96	-	-	-	+	-	-	+	+	+	+	-	-
97	-	-	-	+	-	-	+	+	+	+	-	-
98	-	-	-	+	-	-	-	+	+	+	-	-
99	-	-	+	+	+	+	+	+	+	+	-	-
100	-	-	-	+	+	+	+	+	+	+	-	-
101	+	-	+	+	+	+	+	+	+	+	-	-
102	+	-	+	+	+	+	+	+	+	+	-	-
103	+	-	+	+	+	+	+	+	+	+	-	-
104	-	+	+	+	+	+	+	+	-	+	-	-
105	+	-	+	+	+	+	+	+	+	+	-	-
106	+	-	+	+	+	+	+	+	+	+	-	-
107	+	-	+	+	+	+	+	+	+	+	-	-
108	-	+	+	+	+	+	+	+	-	+	-	-
109	-	-	-	+	-	-	-	-	+	+	-	-
110	-	-	+	+	+	+	-	+	-	+	-	-
111	+	+	+	+	+	+	+	+	-	+	-	-
112	+	-	+	+	+	-	+	+	+	+	-	-
113	+	-	+	+	-	-	+	+	+	+	-	-
114	+	-	+	+	+	-	-	+	-	+	-	-
115	+	+	-	+	+	+	+	+	+	+	-	-
116	+	-	+	+	+	+	+	+	+	+	-	-
117	+	+	-	+	+	+	+	+	+	-	+	+
118	+	+	-	+	+	+	+	+	+	-	+	+
119	+	+	-	+	+	-	-	+	+	-	+	+
120	+	+	-	+	+	+	-	-	+	-	+	+

Table 3 continued ...

OTU #	TEST #																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
121	-	+	+	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-
122	-	+	+	-	+	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-
123	-	+	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
124	-	+	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-
125	-	+	+	-	+	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-
126	-	+	+	-	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-
127	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	+
128	-	+	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+
129	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
130	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-
131	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
132	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
133	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
134	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
135	-	+	+	-	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-
136	-	+	-	-	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-
137	-	+	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
138	-	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
139	-	+	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
140	-	+	+	-	+	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-
141	-	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-
142	-	+	+	-	-	-	-	+	-	-	-	+	-	+	+	+	-	-	-	+
143	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	+	-
144	-	+	+	-	-	-	+	+	-	+	-	+	-	+	+	+	-	-	+	-
145	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-
146	-	+	+	-	-	-	-	+	+	-	-	+	-	+	+	+	-	-	-	+
147	+	+	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+	+
148	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
149	-	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-

Table 3 continued ...

OTU #	TEST #																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
121	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	-
122	-	+	+	+	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-
123	-	+	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	-
124	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-
125	-	+	+	+	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-
126	-	-	+	+	+	-	-	-	-	+	-	+	+	+	+	+	-	+	+	-
127	-	-	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-
128	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
129	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
130	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
131	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	+	+	-
132	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	-	+	+	-
133	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
134	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
135	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
136	-	+	+	+	+	-	-	-	-	+	-	-	+	-	-	+	+	+	+	-
137	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
138	-	-	+	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	+	-
139	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-
140	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-
141	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-
142	-	-	+	+	+	-	+	-	-	+	-	+	-	-	-	-	+	+	-	-
143	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	+	+	-
144	-	-	-	+	+	-	-	-	-	+	-	-	+	-	-	-	+	+	+	-
145	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-
146	-	-	+	+	+	+	+	+	-	+	-	+	+	-	-	-	+	+	-	-
147	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-
148	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+
149	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	-

Table 3 continued ...

DTU #	TEST #																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
121	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
122	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
123	+	+	-	-	+	+	-	-	-	+	-	-	+	-	+	+	+	+	+	+
124	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
125	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
126	-	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
127	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
128	-	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
129	+	+	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+
130	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+
131	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+
132	-	+	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	+	+	+
133	-	+	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	+	-	+
134	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	+	-	+
135	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	+	-	+
136	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
137	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	+	-	+
138	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	+
139	+	-	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+
140	+	+	+	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
141	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	+	-	-
142	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-
143	-	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+
144	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-
145	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-
146	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-
147	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-
148	+	+	-	-	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+
149	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+

Table 3 continued ....

OTU #	TEST #																		
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
121	-	+	+	+	+	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+
122	-	+	+	+	+	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+
123	-	+	+	+	+	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+
124	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
125	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
126	-	+	+	+	+	-	-	-	+	-	+	-	-	-	-	+	+	+	-	+
127	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+
128	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+
129	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+
130	-	+	+	+	+	-	-	+	-	-	-	+	-	-	-	-	+	+	-	+
131	-	+	+	+	+	+	-	+	+	-	+	-	+	-	-	+	+	+	+	+
132	-	+	+	+	+	-	-	-	-	-	+	+	-	-	-	+	+	+	+	+
133	-	+	+	+	+	-	-	+	+	-	+	-	-	-	-	-	-	+	-	+
134	-	+	+	+	+	-	-	+	+	-	-	+	-	-	-	-	-	+	-	+
135	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+
136	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+
137	-	+	+	+	-	-	-	+	+	-	-	+	-	-	-	-	-	+	-	+
138	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+
139	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-	+
140	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+
141	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
142	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
143	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+
144	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
145	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
146	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
148	-	+	+	-	+	-	-	+	+	+	-	+	+	-	+	+	-	+	-	+
149	-	+	+	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-

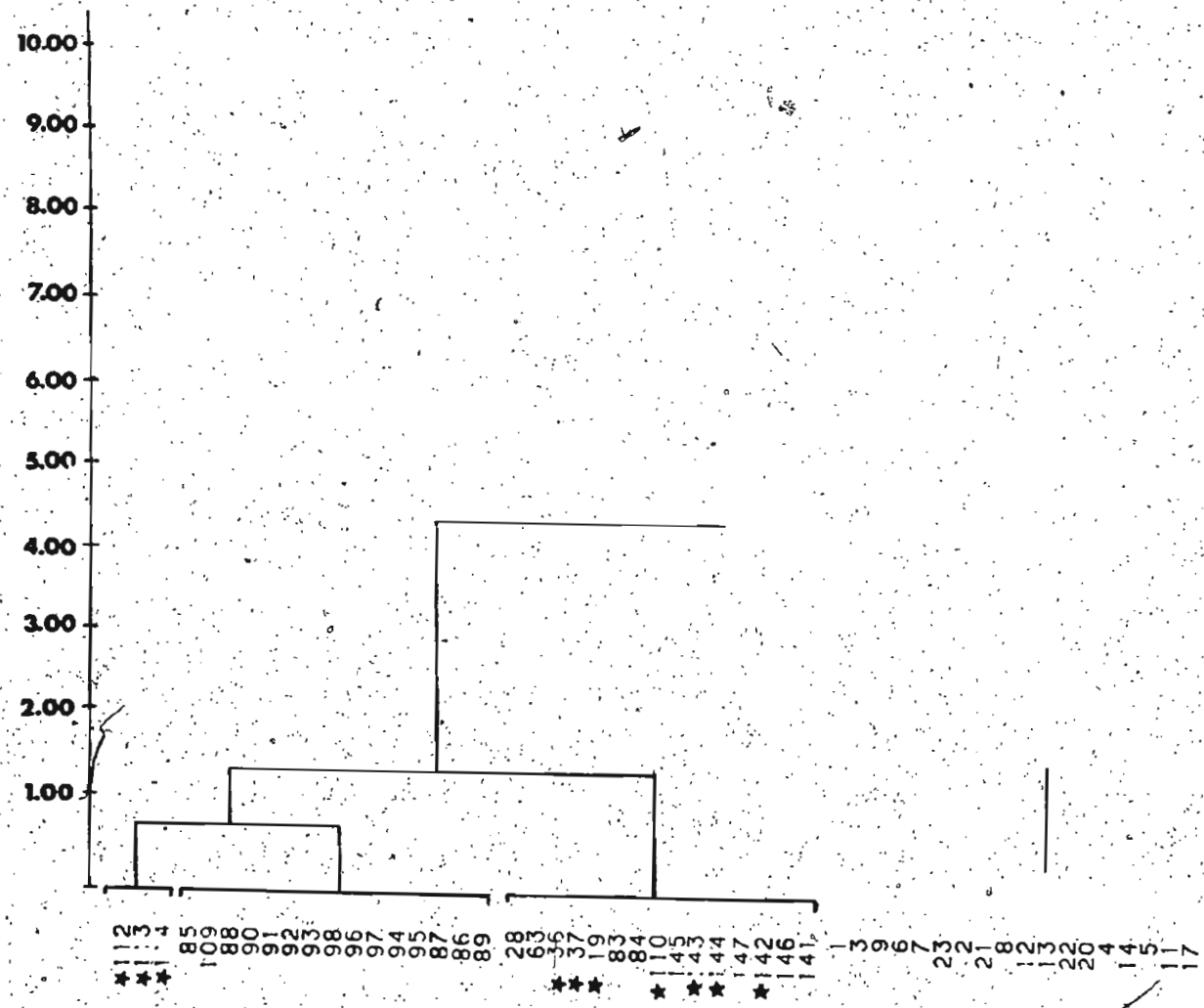
Table 3 continued ...

OTU #	TEST #																			
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
121	+	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+
122	+	-	+	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+
123	+	-	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+	+	+	+
124	+	=	+	=	=	=	+	+	+	+	=	+	=	=	=	=	+	+	+	+
125																				
126	+	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	+	+	+	-
127	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	+
128	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	+
129	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+	+	+	+
130	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+
131	-	-	+	-	-	-	+	+	+	+	+	-	+	-	-	-	-	+	+	+
132	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+
133	-	-	+	-	-	-	+	+	+	+	+	-	+	-	-	-	-	+	+	+
134	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+
135	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+
136	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+
137	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+
138	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+
139	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+
140	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+
141	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
142	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
143	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
144	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
145	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
146	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-
147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
148	-	-	+	+	-	-	+	-	-	+	-	-	-	+	-	-	-	+	+	+
149	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+

Table 3 continued ...

OTU #	TEST #											
	101	102	103	104	105	106	107	108	109	110	111	112
121	+	+	+	+	+	+	+	+	+	-	+	+
122	+	-	+	+	+	+	+	+	+	-	+	-
123	+	+	+	+	+	+	+	+	+	-	+	+
124	+	-	+	+	+	+	+	+	+	-	+	-
125	+	-	+	+	+	+	+	+	+	-	+	-
126	+	+	+	+	+	+	+	+	+	-	+	+
127	+	-	+	+	+	+	-	+	+	-	+	-
128	+	-	+	+	+	+	-	+	+	-	+	-
129	+	-	+	+	+	+	+	+	+	-	+	-
130	+	-	+	+	+	+	+	+	+	-	+	+
131	+	+	+	+	+	+	+	+	+	-	+	+
132	+	-	+	+	+	+	-	+	+	-	+	+
133	+	+	+	-	-	-	-	+	+	-	+	+
134	+	-	+	-	-	-	-	+	+	-	+	+
135	+	-	+	-	+	+	-	+	+	-	+	+
136	+	-	+	+	+	+	+	+	+	-	+	-
137	+	-	+	+	+	+	-	+	+	-	+	+
138	+	-	+	+	+	+	-	+	+	-	+	+
139	+	-	+	+	+	+	+	+	+	-	+	+
140	+	-	+	+	+	+	+	+	+	-	+	+
141	-	-	+	-	-	-	-	+	+	-	+	-
142	-	-	-	-	+	+	+	+	-	-	+	-
143	-	-	-	-	+	+	-	+	+	+	-	-
144	-	-	-	+	+	+	+	+	+	+	-	-
145	+	-	-	+	+	-	-	-	-	+	-	-
146	+	+	+	-	-	-	+	+	-	-	+	-
147	-	-	-	-	-	-	-	-	-	+	-	-
148	+	-	+	+	+	+	-	+	+	-	+	+
149	+	+	+	+	+	+	-	-	+	-	+	+

Figure 1. Dendrogram produced by the Euclidean distance coefficient with relocate clustering. This analysis used 149 OTU and 112 characters. The Y-axis gives dissimilarity values at which OTU and clusters of OTU merge. The asterisk indicates OTU which were relocated during the analysis.



EUCLIDEAN DISTANCE COEFFICIENT, RELOCATE CLUSTERING

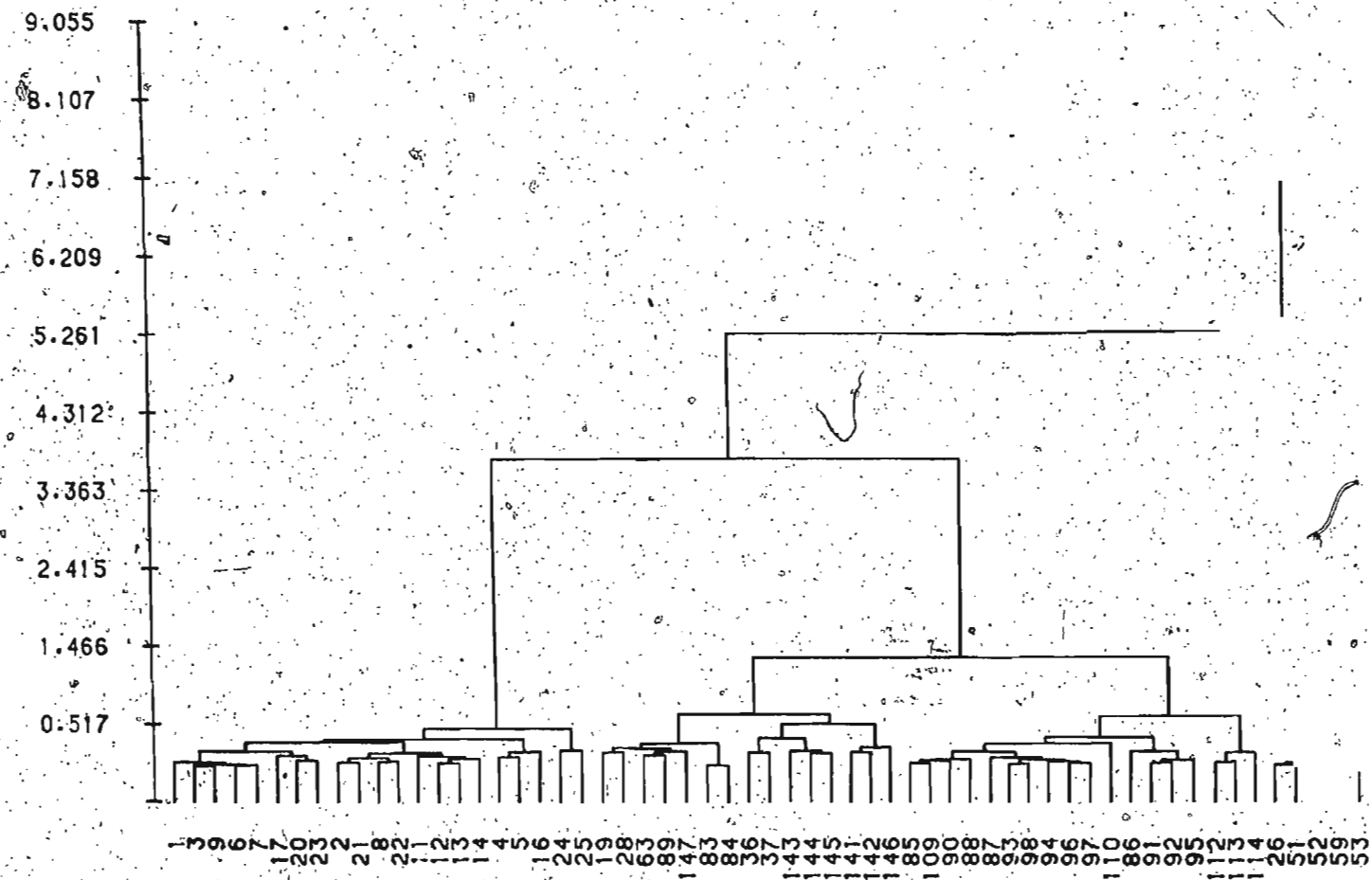
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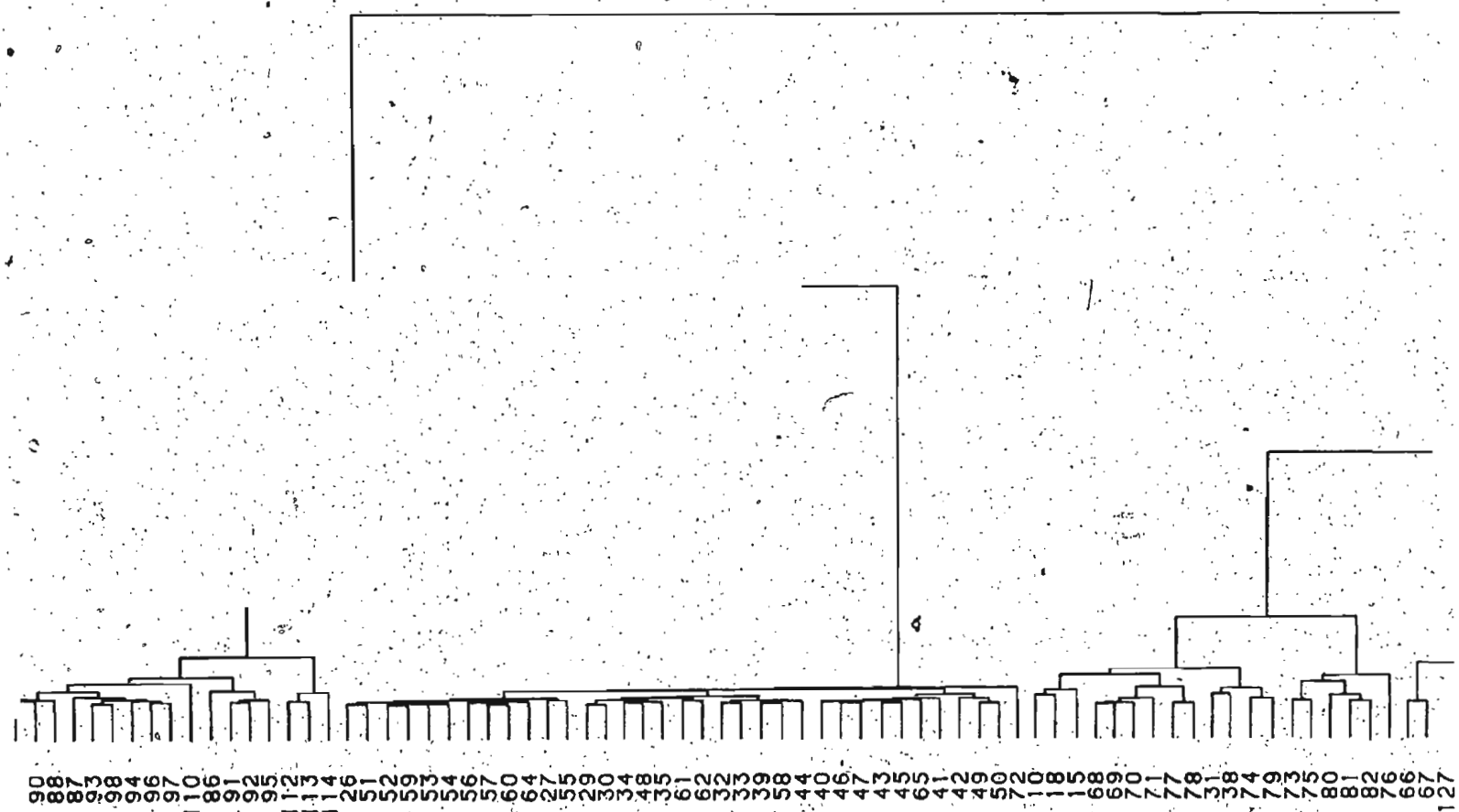
2 of 2

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Figure 2. Dendrogram produced by the Euclidean distance coefficient with Ward's clustering. This analysis used 149 OTU and 112 characters. The Y-axis gives the dissimilarity values at which OTU and clusters of OTU merge.



EUCLIDEAN DISTANCE COEFFICIENT WARD'S CLUSTERING METHOD



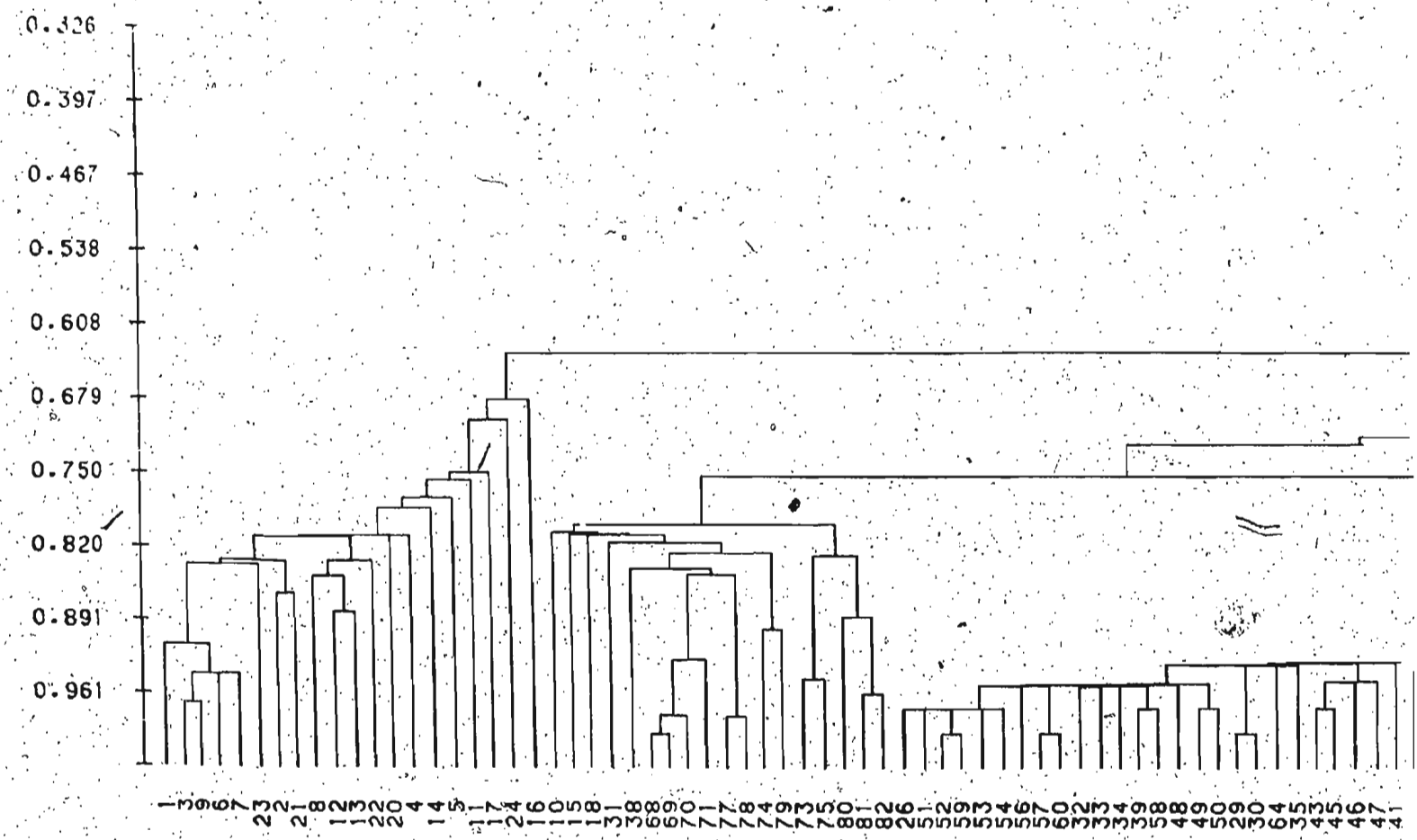
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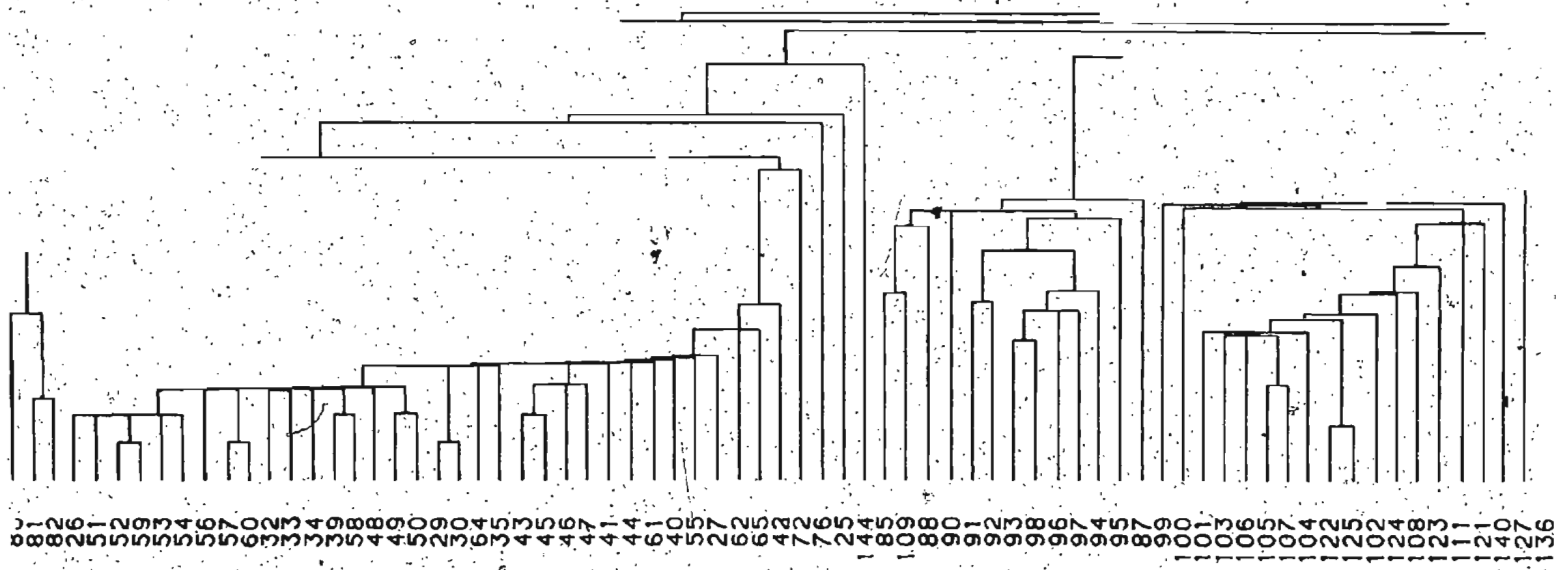
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Figure 3. Dendrogram produced by the Jaccard coefficient with single linkage clustering. This analysis used 149 OTU and 112 characters. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.



JACCARDS COEFFICIENT. SINGLE LINKAGE CLUSTERING METHOD



ING METHOD

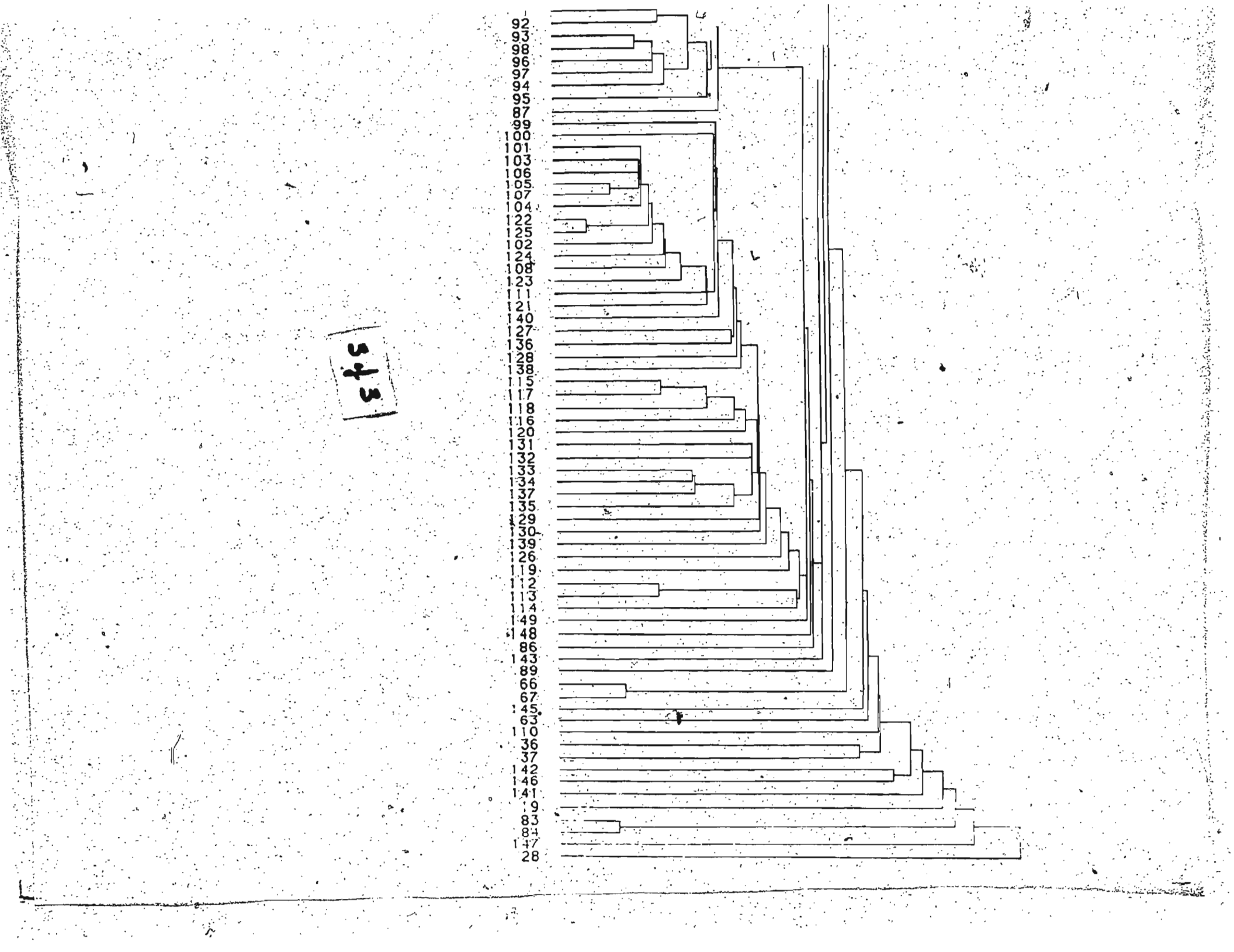
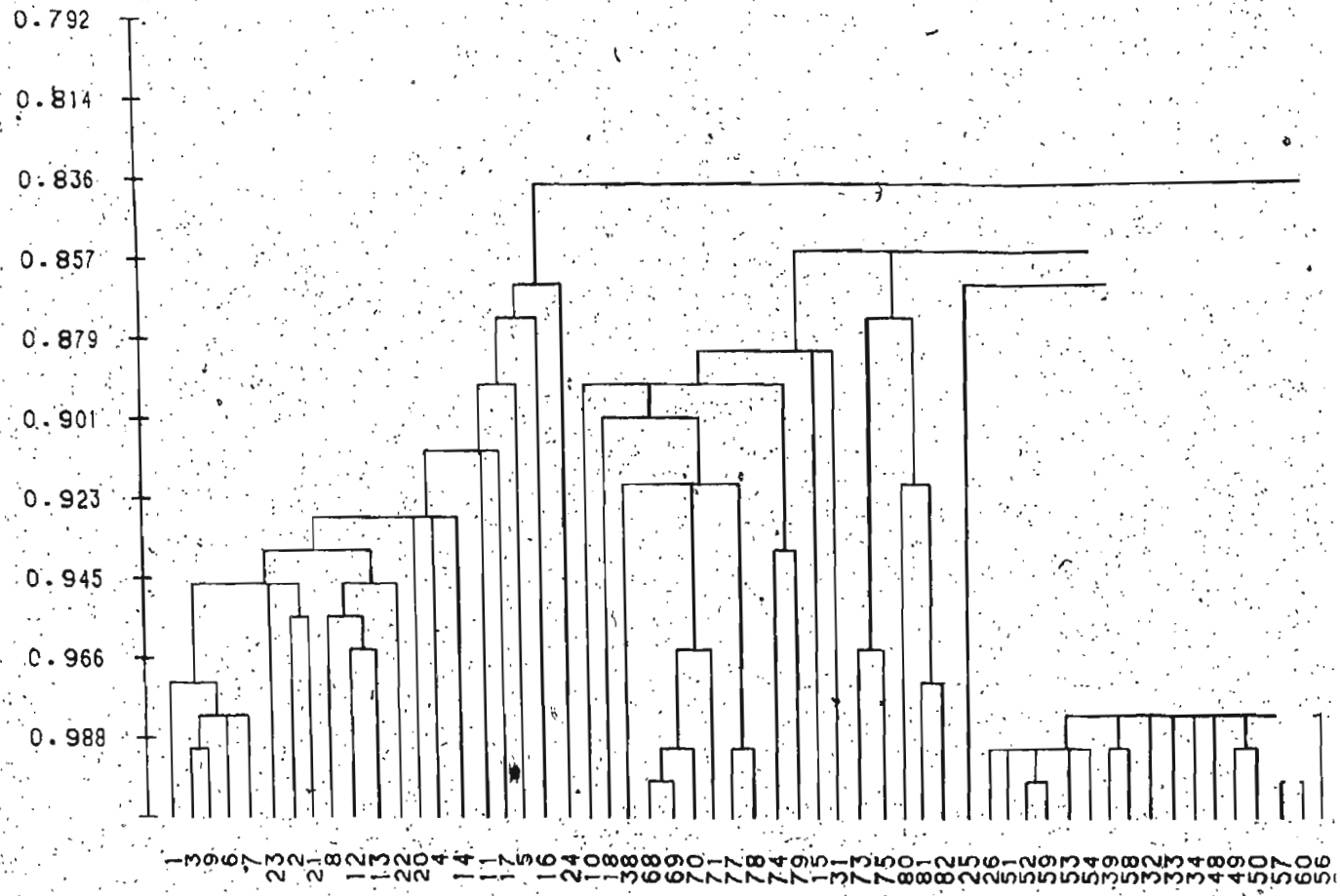
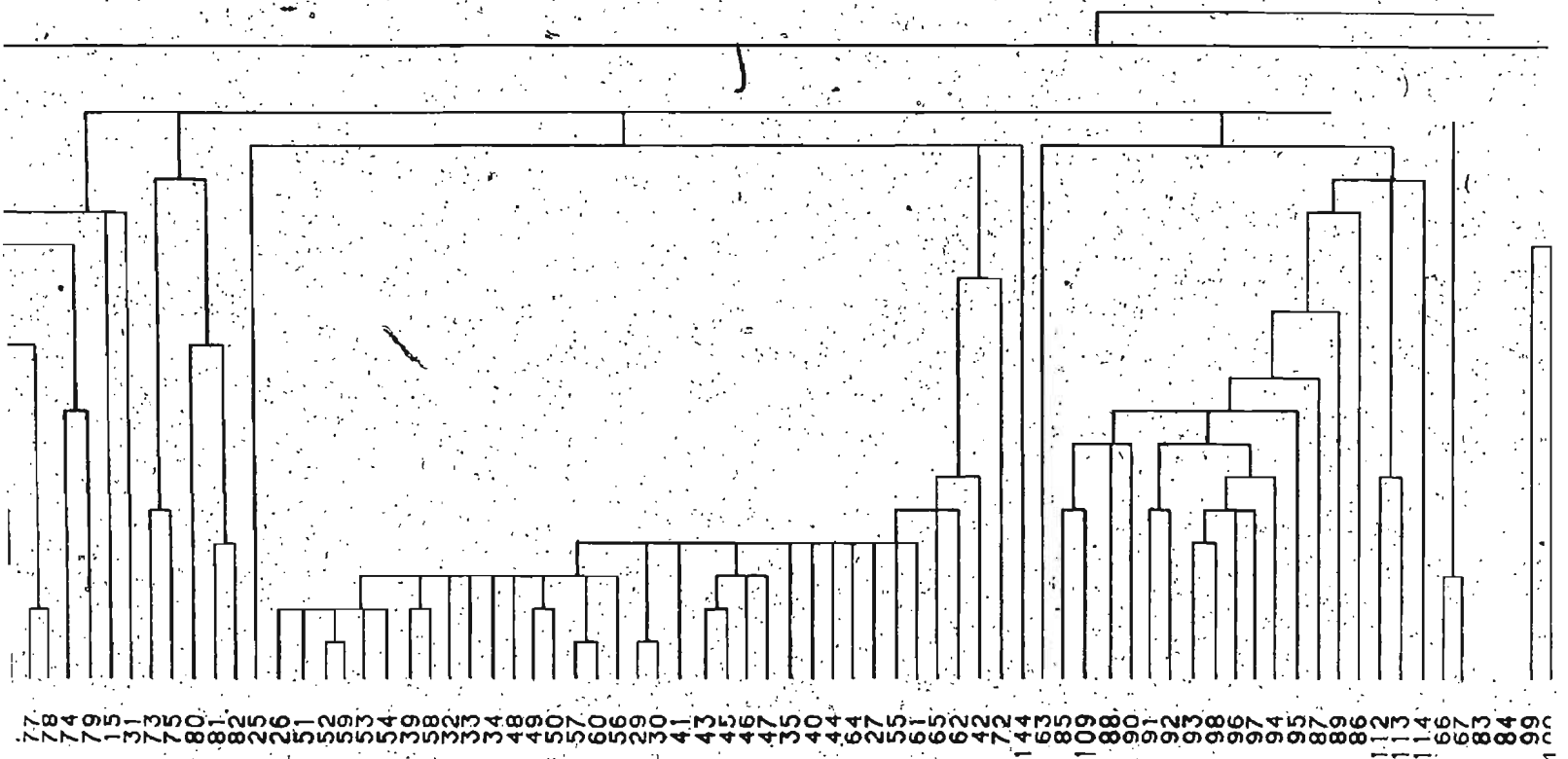

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Figure 4. Dendrogram produced by the simple matching coefficient with single linkage clustering. This analysis used 149 OTU and 112 characters. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.



SIMPLE MATCHING COEFFICIENT SINGLE LINKAGE CLUSTERING



LE LINKAGE CLUSTERING

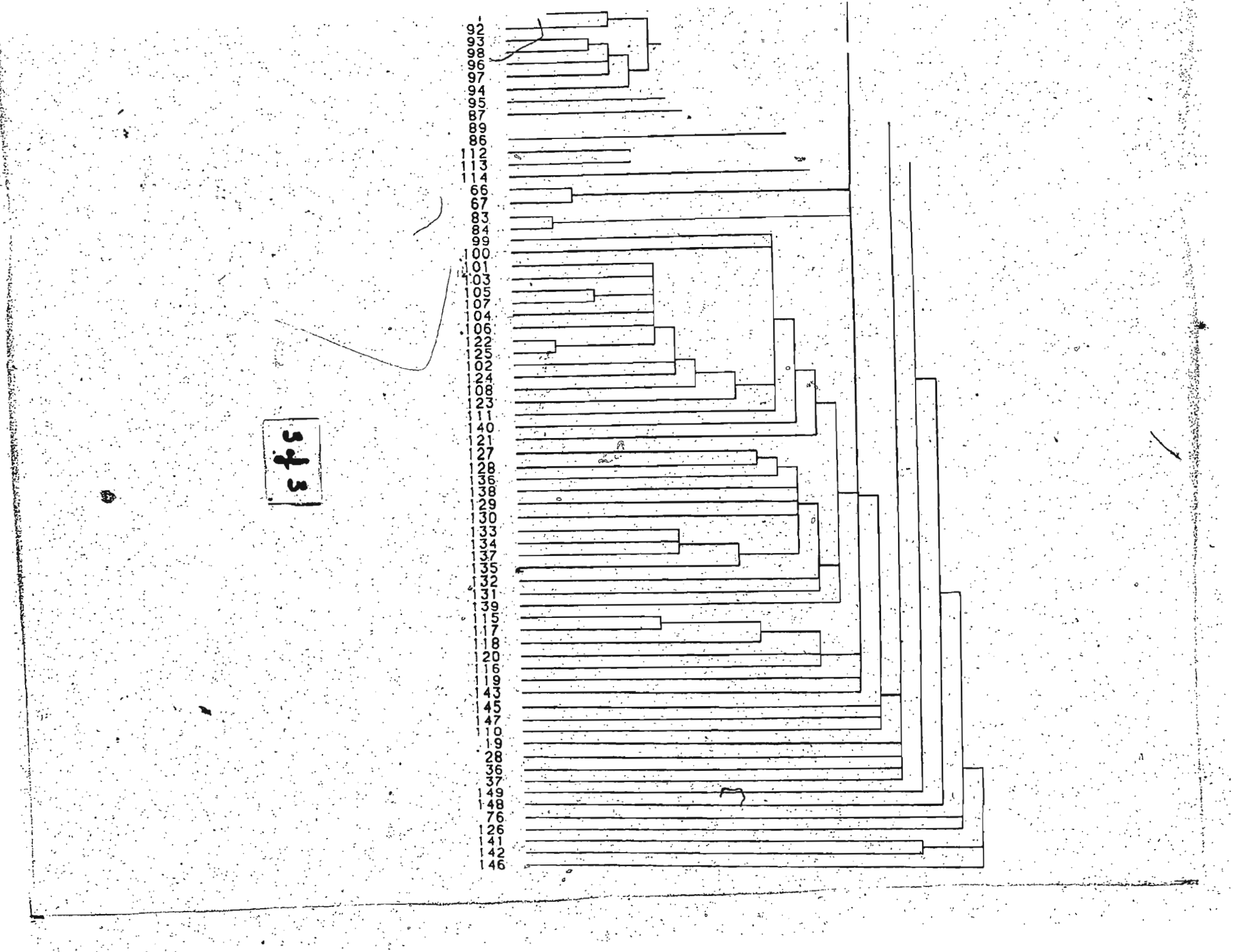
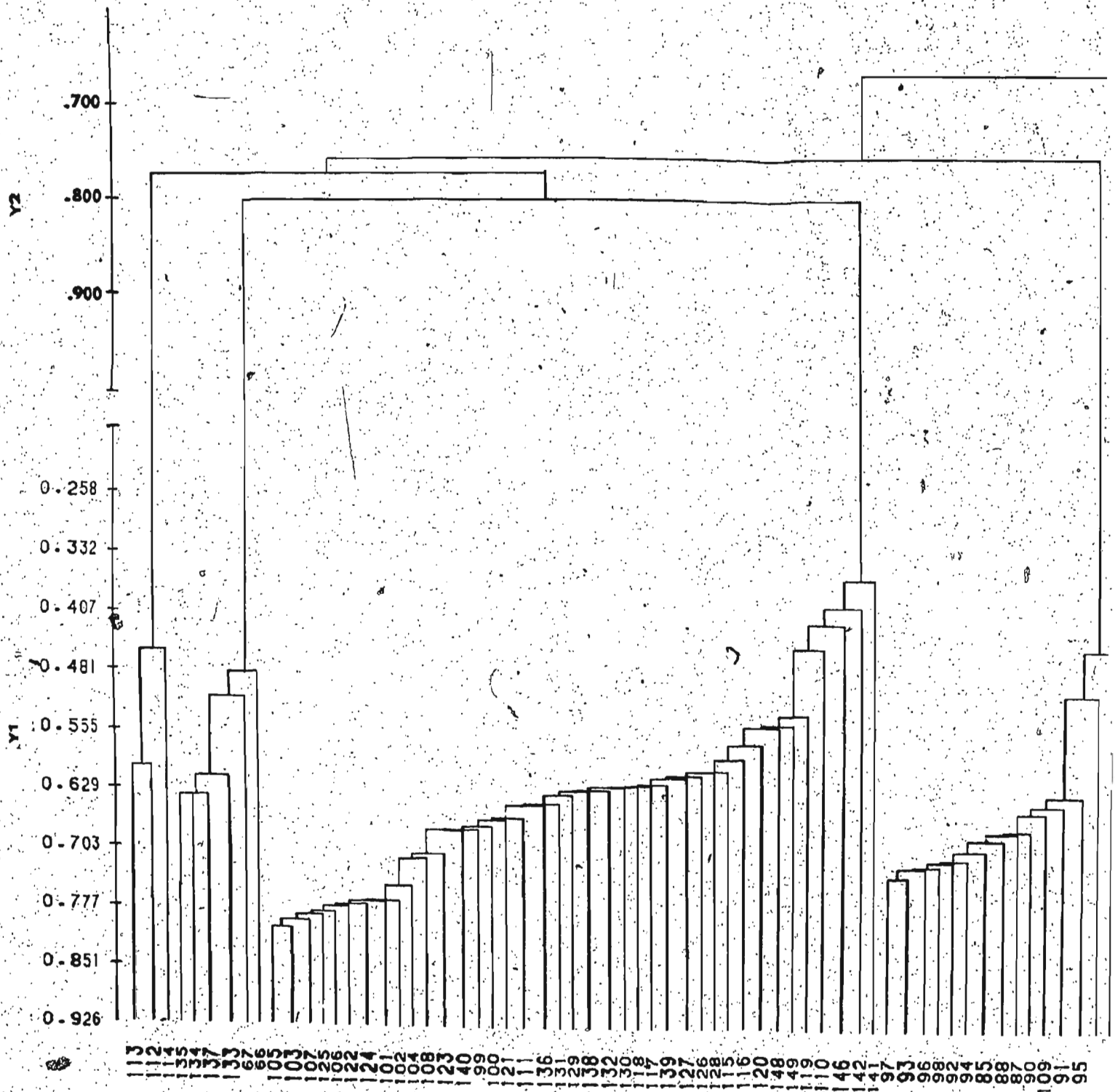

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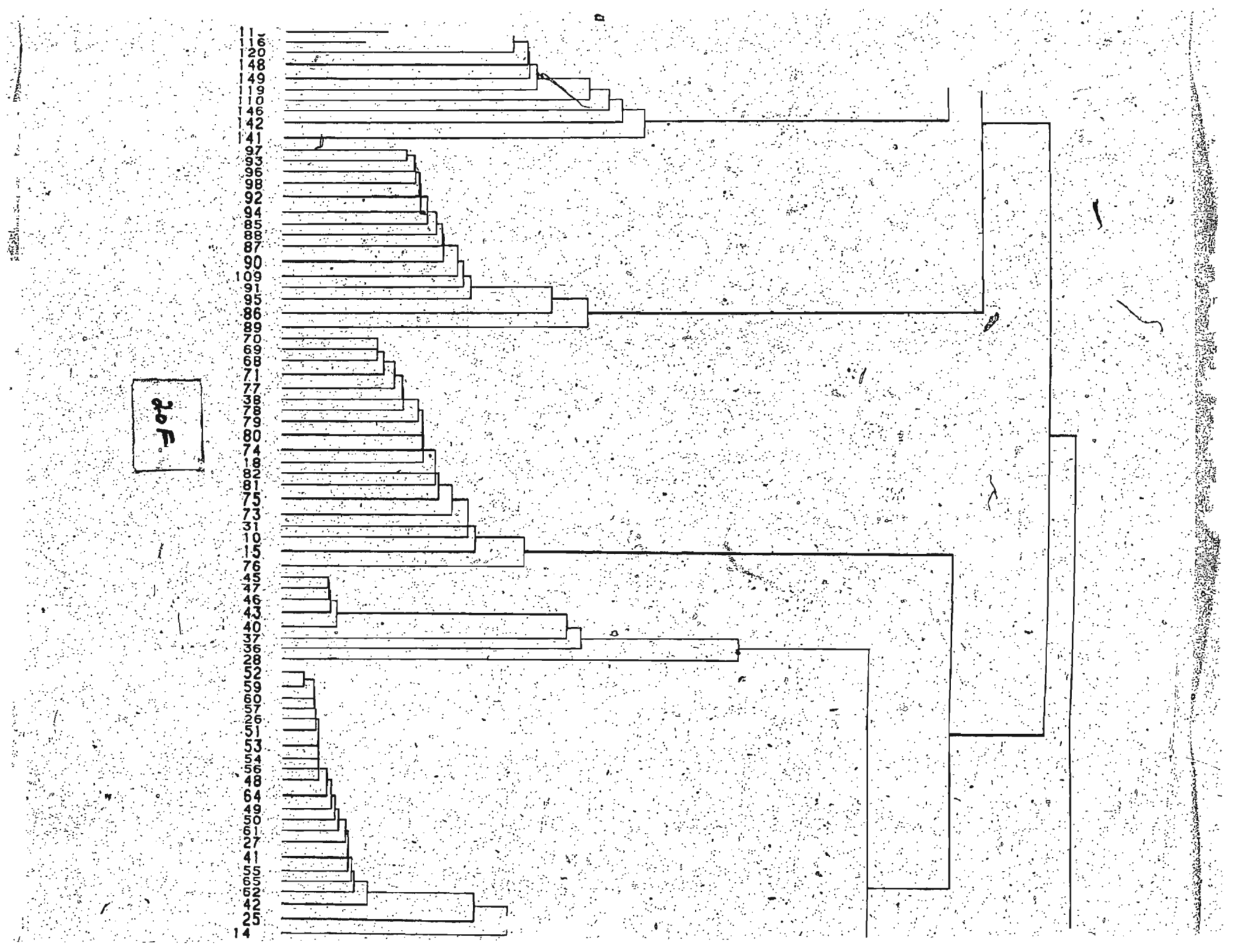
Figure 5. Dendrogram produced by the Jaccard coefficient with density clustering. This analysis used 149 OTU and 112 characters. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.

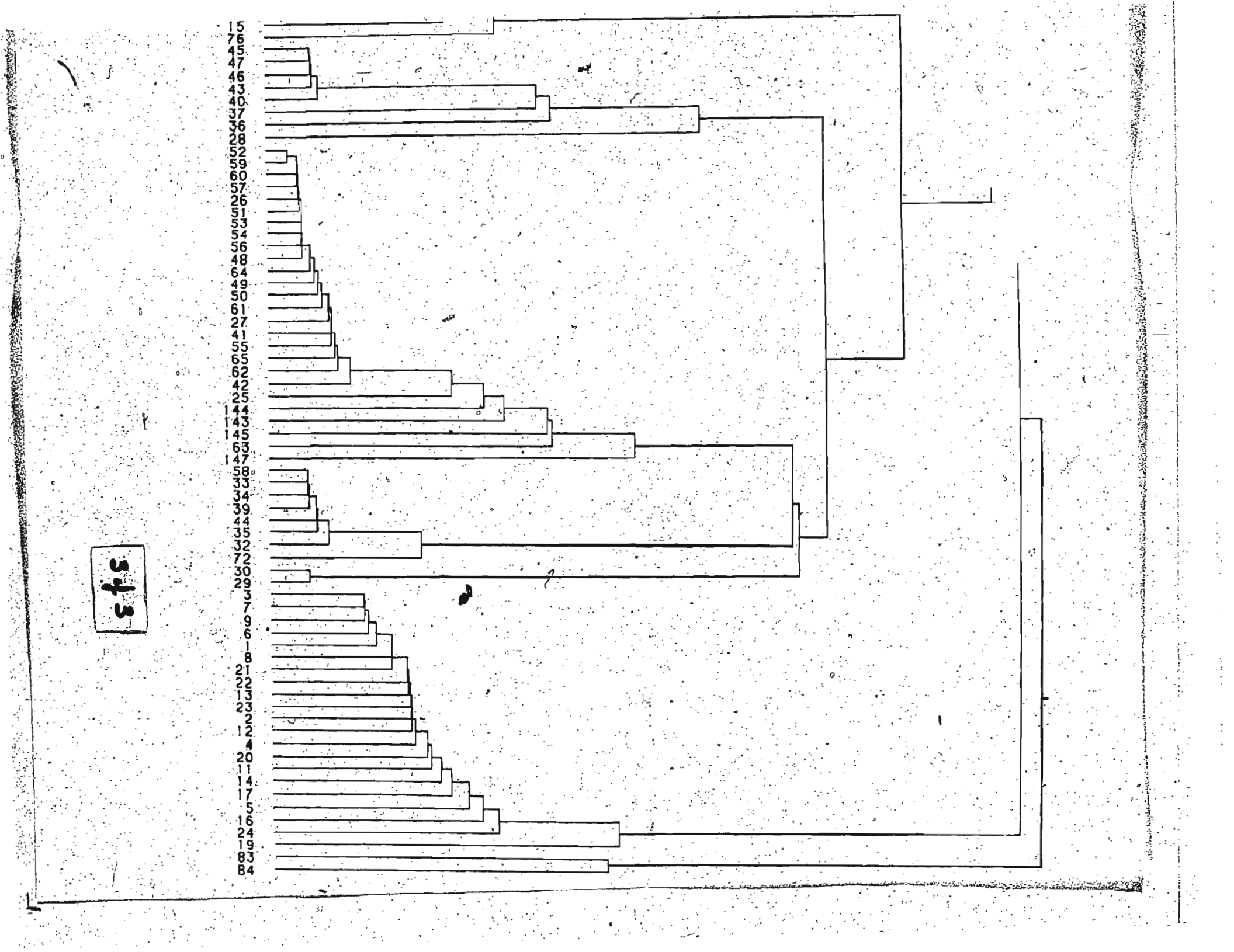
Y1 = density clustering

Y2 = single linkage clustering



JACCARDS COEFFICIENT, DENSITY CLUSTERING


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APPENDIX B

Table 1. Tests used for identifying fermentative marine bacteria (Baumann and Baumann, 1981). Numbers correspond to data matrix.

1. PHB accumulation
2. arginine dihydrolase
3. luminescence
4. acetoin/diacetyl production
5. growth at 4°C
6. growth at 35°C
7. growth at 40°C
8. amylase
9. lipase
10. gelatinase
11. D-xylose
12. L-arabinose
13. D-galactose
14. sucrose
15. maltose
16. cellobiose
17. D-gluconate
18. D-glucuronate
19. acetate
20. propionate
21. butyrate
22. heptanoate
23. pelargonate
24. DL-lactate
25. α-ketoglutarate
26. sorbitol
27. inositol
28. ethanol
29. p-hydroxybenzoate
30. glycine
31. D-α-alanine
32. L-serine
33. L-leucine
34. L-glutamate
35. L-arginine
36. L-citrulline
37. γ-aminobutyrate
38. δ-aminovalerate
39. L-proline
40. putrescine
41. sarcosine

TABLE 2. Data matrix for fermentative strains.

Reference strains are named.

OTU #	TEST #																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	+	-	-	-	+	-	-	-	+	+	+	+	-	+	+	+	-	+	-	+	+
2	-	-	-	-	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+
3	+	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+
4	+	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+
5	+	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+
6	+	-	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	+	-	+	+
7	+	-	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	+	-	+	+
8	+	-	-	-	+	-	-	-	+	+	+	-	-	+	+	+	-	+	-	+	+
9	+	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+
10	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	+	+	+	+
11	+	-	-	-	+	+	+	-	+	+	+	-	-	+	+	+	-	+	-	+	+
12	+	-	-	-	+	-	-	-	+	+	+	-	-	+	+	+	-	+	-	+	+
13	-	-	-	-	+	-	-	-	+	+	+	-	-	+	+	+	-	+	-	+	+
14	+	-	-	-	+	-	-	-	+	+	+	-	-	+	+	-	-	+	-	+	-
15	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+
16	-	-	-	-	+	-	-	-	+	+	+	-	-	+	+	-	-	+	-	+	+
17	-	-	-	-	+	-	-	+	+	+	+	+	-	+	+	-	-	+	-	+	+
18	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	+	-	+	+
19	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-
20	-	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+
21	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+	-	+	-	+	+
22	-	-	+	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	-	+	+
23	-	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+
24	-	-	-	-	+	-	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+
25	-	+	-	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+
26	-	+	-	-	+	-	-	+	+	+	-	-	-	+	-	+	+	+	-	+	+
27	-	+	-	-	+	-	-	+	-	+	-	-	-	+	-	+	+	+	-	+	+
28	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
29	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
30	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
31	+	+	-	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+
32	-	-	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
33	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
34	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
35	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
36	+	-	-	-	+	+	+	-	-	-	+	-	+	-	-	-	-	+	+	+	+
37	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+
38	+	-	-	-	+	+	+	+	-	+	+	-	-	+	-	-	-	+	+	+	+
39	-	-	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
40	+	+	-	-	+	-	-	-	+	+	+	-	-	+	-	+	+	+	-	+	+

TABLE 2 continued...:

OTU #	TEST #																				
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-
2	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-
3	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-
4	-	-	+	-	-	-	+	-	+	-	+	-	-	+	+	+	+	-	+	-	-
5	-	-	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+	-	+	-	+
6	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-
7	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-
9	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-
10	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	-	+	-	+
11	-	-	+	-	-	-	+	-	+	-	-	-	-	+	+	+	+	-	+	-	-
12	-	-	+	-	-	-	-	-	+	-	+	-	-	+	+	+	-	-	+	-	-
13	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-
14	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-	-	+	-	-
15	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+
16	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+
17	-	-	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	-	-	+
18	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+
19	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
20	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-
21	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-
22	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-
23	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-
24	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+	-	+	+	-	-
25	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	-
26	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
27	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
29	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
30	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
31	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+
32	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
33	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
34	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
35	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
36	+	-	-	+	-	+	-	-	-	-	+	+	-	+	+	+	-	-	+	-	-
37	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
38	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+
39	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
40	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-

TABLE 2 continued....

OTU #	TEST #																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
41	+	-	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
42	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	+	+	-	+	+	+
43	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	+	+	+	-	+	+
44	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	+	+	+	-	+	+
45	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	+	+	+	-	+	+
46	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	+	+	+	-	+	+
47	-	-	-	-	+	-	-	-	+	+	+	-	-	+	-	+	+	+	-	+	+
48	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
49	-	+	-	-	+	-	-	+	+	+	-	-	-	+	-	+	+	-	+	+	+
50	+	+	-	-	+	-	-	+	+	+	-	-	-	+	-	+	+	-	+	+	+
51	+	+	-	-	+	-	-	+	+	+	-	-	-	+	-	+	+	+	-	+	+
52	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
53	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
54	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	-	-	+	+
55	+	+	-	-	+	-	-	-	-	+	-	-	-	+	-	+	+	-	-	+	+
56	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
57	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
58	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
59	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
60	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
61	-	+	-	-	+	-	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+
62	-	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+
63	+	+	-	-	+	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	-
64	+	+	-	-	+	+	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+
65	-	+	-	-	+	-	-	-	+	-	+	-	-	+	+	+	+	-	-	+	+
85	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	+	+
86	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	+	+
87	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	+	+
88	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	+	+
89	+	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-
90	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	+
91	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+
92	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	+
93	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
94	+	-	-	-	+	-	-	-	+	+	-	+	-	-	+	+	-	-	-	+	+
95	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+
96	+	-	-	-	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+
97	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
98	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
99	+	-	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	+	+

TABLE 2 continued....

OTU #	TEST #																				
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
41	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
42	-	-	-	+	+	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-
43	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
44	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
45	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
46	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
47	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
48	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
49	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
50	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
51	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
52	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
53	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
54	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
55	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
56	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	+	-	-	+	-	-
57	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
58	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
59	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
60	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
61	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
62	-	-	-	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-
63	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
64	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
65	-	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-
85	+	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
86	+	-	-	+	+	-	+	-	-	-	+	+	+	+	-	-	+	-	+	-	-
87	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-
88	+	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
89	+	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-
90	+	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-
91	+	-	-	+	+	-	-	-	-	-	+	-	+	+	-	-	+	-	+	-	-
92	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-
93	+	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-
94	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-
95	+	-	-	+	+	-	-	-	-	-	+	-	+	+	-	-	+	-	+	-	-
96	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-
97	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-
98	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-
99	+	-	-	+	+	-	-	+	-	+	+	+	+	+	+	-	+	-	+	-	-

TABLE 2 continued....

OTU #	TEST #																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
100	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	+	+	+	+
101	+	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+	+
102	-	-	-	-	+	-	-	+	+	+	-	-	+	-	+	+	-	+	+	+	+
103	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	+	+	+	+
104	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	+	+	+	+
105	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	+	+	+	+
106	-	-	-	-	+	-	-	-	+	+	-	+	-	-	+	+	-	+	+	+	+
107	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	+	-	+	+	+	+
108	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	+	+	+	+
109	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
110	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	+
111	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+
112	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
113	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
114	-	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
115	-	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	+	+	+	+
116	-	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-	-	+	+	+	+
V FISC	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-
V LOGE	-	-	+	-	+	-	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-
P LEIO	+	-	+	+	-	+	-	-	+	-	-	-	-	+	-	-	-	+	-	+	-
P ANGU	+	-	-	-	+	+	-	-	+	+	-	+	-	+	+	+	-	+	-	+	-
P PHOS	+	-	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-
V HARV	-	-	+	-	-	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+
V CAMP	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	-	-	+	+
V PARA	-	-	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	+	+	+	+
V ALGI	-	-	-	+	-	+	+	+	+	+	-	-	-	-	+	+	-	+	-	+	+
V NATR	+	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
V VULN	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	+
V SPL1	-	+	+	-	-	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
V SPL2	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-
V PEL1	-	-	-	-	-	+	-	-	+	-	+	-	-	+	+	+	-	+	-	+	+
V PEL2	-	-	-	-	-	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+	+
V NERE	+	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	+	+
V ANG1	-	+	-	+	-	+	-	+	+	+	-	-	+	+	+	+	+	+	-	+	+
V ANG2	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	-
V NIGR	+	-	-	-	-	-	-	+	+	+	-	-	-	+	-	+	+	+	+	+	+
V PROT	-	+	-	+	-	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	-
E-3	+	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	+	+	-	+	+

TABLE 2 continued....

OTU #	TEST #																					
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
100	+	-	-	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-
101	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+
102	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+
103	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+
104	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+
105	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+
106	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+
107	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+
108	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	-
109	+	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
110	+	-	-	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+
111	+	+	-	+	+	-	-	+	-	-	+	+	+	+	-	-	+	+	+	-	+
112	+	+	-	+	+	-	-	+	-	-	-	+	+	+	-	-	-	+	-	-	-
113	+	+	-	+	+	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-
114	+	+	-	+	+	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-
115	-	+	+	+	+	-	+	-	+	-	+	+	+	+	-	-	+	-	+	-	+
116	-	+	+	+	+	-	+	-	+	-	+	+	+	+	-	-	+	-	+	-	+
V FISC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
V LOGE	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
P LEIO	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-
P ANGU	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
P PHOS	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V HARV	-	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-
V CAMP	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-
V PARA	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	-	-	-	+	+	-
V ALGI	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	-	-	-	+	+	-
V NATR	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
V VULN	-	-	-	+	+	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-
V SPL1	-	+	-	+	+	-	-	-	-	+	+	+	-	+	+	+	-	-	+	-	-
V SPL2	-	-	-	+	+	-	-	-	-	+	+	+	-	+	-	-	-	-	+	-	-
V PEL1	-	-	-	+	-	-	-	+	-	+	+	+	-	+	+	+	-	-	+	+	-
V PEL2	-	-	-	+	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	-
V HERE	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-
V ANG1	-	-	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	+	-	-
V ANG2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-
V NIGR	-	+	-	+	+	+	+	+	-	+	+	+	-	+	-	-	-	-	+	-	-
V PROT	-	-	+	+	+	+	-	-	-	+	+	+	-	+	+	-	-	-	+	+	-
E-3	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+

Table 3. Tests used for identifying oxidative marine bacteria which accumulate PHB (Baumann and Baumann, 1981). Numbers correspond to data matrix.

1. oxidase	15. mannitol
2. growth at 4°C	16. glycerol
3. growth at 35°C	17. ethanol
4. D-ribose	18. phenylacetate
5. D-glucose	19. quinate
6. D-galactose	20. glycine
7. D-fructose	21. L-threonine
8. D-gluconate	22. L-ornithine
9. N-acetylglucosamine	23. δ-aminovalerate
10. valerate	24. L-histidine
11. pelargonate	25. L-tyrosine
12. glycolate	26. ethanolamine
13. DL-glycerate	27. sarcosine
14. aconitate	28. allantoin

TABLE 4. Data matrix for oxidative, PHB–positive strains.

Reference strains are named.

OTU #	TEST #													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
68	+	+	+	+	+	+	+	+	+	+	+	–	+	+
69	+	+	+	+	+	+	+	+	+	+	+	–	+	+
70	+	+	+	+	+	+	+	+	+	+	+	–	+	+
71	+	+	+	+	+	+	+	+	+	+	+	–	+	+
73	+	+	+	+	+	+	+	+	+	+	–	+	+	+
74	+	+	+	+	+	+	+	+	+	+	+	–	+	+
75	+	+	+	+	+	+	+	+	+	+	+	+	+	+
76	+	+	–	+	+	+	–	+	+	+	–	–	–	–
77	+	+	+	+	+	–	+	+	+	+	+	+	+	+
78	+	+	+	+	+	–	+	+	+	+	+	+	+	+
79	+	+	+	+	+	+	+	+	+	+	+	+	+	+
80	+	+	–	+	+	+	+	+	+	+	+	+	+	+
81	+	+	–	+	+	+	+	+	+	+	+	+	+	+
82	+	+	–	+	+	+	+	+	+	+	+	+	+	+
P DOUD	+	–	+	+	–	–	+	–	–	–	–	+	+	+
P MARI	–	+	+	+	+	+	+	+	–	+	+	–	+	+
B-2	+	–	+	–	+	+	+	+	+	+	–	+	+	+
I-2	+	–	+	–	+	–	–	–	–	+	–	–	–	–
H-1	+	–	+	–	+	–	+	–	–	–	–	–	–	+
I-1	+	–	–	–	–	–	–	–	–	+	–	–	–	–
G-1	+	–	+	–	–	–	–	–	–	+	+	–	–	+

TABLE 4 continued....

OTU #	TEST #													
	15	16	17	18	19	20	21	22	23	24	25	26	27	28
68	+	+	+	-	-	-	-	+	-	-	+	-	+	-
69	+	+	+	-	-	-	-	+	-	-	+	-	+	-
70	+	+	+	-	-	-	-	+	-	-	+	-	+	-
71	+	+	+	-	-	+	-	+	-	-	+	-	+	-
73	+	+	+	+	-	+	-	+	+	-	+	-	+	+
74	+	+	+	-	-	+	+	+	-	-	+	-	+	-
75	+	+	+	+	-	+	-	+	+	-	+	-	+	+
76	+	+	+	-	+	+	+	-	+	-	+	-	-	-
77	+	+	+	+	+	+	-	+	-	-	+	-	+	-
78	+	+	+	+	+	+	-	+	-	-	+	-	+	-
79	+	+	+	+	+	+	+	+	-	-	+	-	+	-
80	+	+	+	+	+	+	+	+	+	-	+	-	+	-
81	+	+	+	+	+	+	-	+	+	-	+	-	+	+
82	+	+	+	+	+	+	-	+	+	-	+	-	+	+
P DOUG	-	-	+	-	-	+	+	+	+	+	-	-	+	+
P MARI	+	+	+	-	-	-	-	+	-	-	+	-	+	-
B-2	+	+	-	+	+	+	+	+	-	+	+	+	+	+
I-2	-	+	-	-	-	-	+	+	-	+	+	+	+	-
H-1	+	-	+	-	+	-	-	-	-	-	-	+	-	-
I-1	-	-	+	-	-	+	+	+	+	-	-	+	+	-
G-1	-	-	+	+	+	-	-	+	+	-	+	-	-	-

Table 5. Tests used for identifying oxidative marine bacteria which do not accumulate PHB (Baumann and Baumann, 1981). Numbers correspond to data matrix.

1. oxidase
2. denitrification
3. growth at 35°C
4. growth at 40°C
5. amylase
6. gelatinase
7. lipase
8. alginase
9. chitinase
10. D-glucose
11. D-mannose
12. D-galactose
13. sucrose
14. cellobiose
15. melibiose
16. lactose
17. salicin
18. D-gluconate
19. N-acetylglucosamine
20. succinate
21. fumarate
22. DL-malate
23. DL-β-hydroxybutyrate
24. DL-glycerate
25. citrate
26. aconitate
27. erythritol
28. mannitol
29. glycerol
30. ethanol
31. γ-aminobutyrate
32. L-tryosine
33. sorbitol
34. α-ketoglutarate
35. m-hydrozybenzoate

TABLE 6. Data matrix for oxidative, PHB-negative strains.

Reference strains are named.

OTU #	TEST #																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
66	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
67	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
72	+	+	–	–	+	+	+	+	–	+	+	+	–	+	–	–	–	+
83	+	–	–	–	–	+	+	+	–	+	–	–	–	+	–	–	–	–
84	+	–	–	–	–	+	+	+	–	+	–	–	–	+	–	–	–	–
117	+	–	–	–	–	–	+	–	–	+	+	+	–	–	–	–	–	+
118	+	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	+
119	+	–	–	–	–	+	+	–	–	+	–	+	–	–	–	–	–	–
120	+	+	–	–	–	–	+	–	–	+	+	+	–	–	–	–	–	–
121	+	+	+	+	–	+	+	–	–	+	–	+	–	–	–	–	–	+
122	+	+	+	–	+	+	+	–	–	+	–	–	+	–	–	–	–	+
123	+	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	+
124	+	+	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	+
125	+	+	+	–	+	+	+	–	–	+	–	–	+	–	–	–	–	+
126	+	+	+	–	–	+	+	–	–	+	–	+	–	–	–	–	–	+
127	+	–	–	–	–	+	+	–	–	+	–	–	+	–	–	–	–	–
128	+	+	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
129	+	–	–	–	–	–	+	–	–	+	–	–	+	–	–	–	–	+
130	–	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–
131	+	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	+
132	+	–	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	+
133	+	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–
134	+	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
135	+	+	+	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–
136	+	–	+	–	+	+	+	–	–	+	–	–	+	–	–	–	–	–
137	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
138	+	–	+	–	–	+	+	–	–	+	–	+	+	–	–	–	–	+
139	+	+	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	+
140	+	+	+	+	–	+	+	–	–	+	–	–	–	–	–	–	–	+
A COHN	+	–	+	+	–	–	–	–	–	+	+	–	–	–	–	–	–	+
A VAGA	–	–	+	–	–	–	–	–	–	+	+	+	–	+	–	–	–	+
A HACL	+	–	+	+	+	+	+	–	–	+	–	+	+	+	+	+	+	+
A HALD	+	–	+	–	+	+	+	–	+	+	+	–	+	–	–	–	–	–
A ESPE	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	+	–	–
A UNDI	+	–	–	–	+	+	+	–	+	+	–	–	+	–	–	–	–	–
A RUBR	+	–	+	–	+	+	+	–	–	+	+	–	–	–	–	–	–	–
A LUTE	+	–	+	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–
A CITR	+	–	+	+	+	+	+	–	+	+	+	–	–	–	–	–	–	–
P NAUT	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
H-2	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 6 continued....

OTU #	TEST #																	
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
66	+	+	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	
67	+	+	+	-	+	-	-	-	-	-	-	-	+	+	-	+	-	
72	+	+	+	+	-	-	+	+	-	+	+	-	+	-	-	+	-	
83	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
84	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
117	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	
118	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	
119	-	+	+	+	+	-	+	-	-	-	-	-	+	+	+	+	-	
120	-	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	
121	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	
122	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	
123	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	
124	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	
125	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	
126	+	+	+	+	+	-	+	+	-	+	-	+	+	+	-	+	-	
127	+	+	+	+	-	+	+	+	-	-	-	+	+	+	-	+	-	
128	+	+	+	+	-	-	+	+	-	-	-	+	+	+	-	+	-	
129	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	
130	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-	
131	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	
132	-	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	
133	-	+	+	-	+	-	-	-	-	-	-	+	+	+	-	+	-	
134	-	+	+	-	+	-	-	-	-	-	-	+	+	+	-	+	-	
135	-	+	+	+	+	-	+	+	-	-	-	+	+	+	-	+	-	
136	+	+	+	+	+	-	+	+	-	+	-	+	+	+	-	+	-	
137	-	+	+	+	-	-	+	+	-	-	-	+	+	+	-	+	-	
138	+	+	+	+	-	-	+	+	-	-	-	+	+	+	-	+	-	
139	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-	
140	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	
A CONN	-	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	
A VAGA	+	+	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	
A MACL	-	-	-	-	+	+	-	-	-	+	+	+	-	+	-	-	-	
A HALO	+	+	+	-	+	-	+	+	-	+	-	+	-	+	-	-	-	
A ESPE	-	-	-	-	-	-	+	+	-	+	-	-	-	+	-	-	-	
A UNDI	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	
A RUBR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A LUTE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A CITR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
P NAUT	-	+	+	+	+	-	+	+	-	-	-	+	-	-	-	-	-	
H-2	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	