

A NUMERICAL TAXONOMIC STUDY OF  
PELAGIC AND EUBENTHIC BACTERIA  
FROM THE NORTHWEST ATLANTIC  
OCEAN NEAR NEWFOUNDLAND

CENTRE FOR NEWFOUNDLAND STUDIES

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A Numerical Taxonomic Study  
of Pelagic and Epibenthic Bacteria  
from the Northwest Atlantic Ocean  
Near Newfoundland

by

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

Department of Biology  
Memorial University of Newfoundland

October, 1984

St. John's

Newfoundland

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ISBN 0-315-31036-7

#### ABSTRACT

An examination of bacterial strains from the pelagic and epibenthic regions from the water column in the Northwestern Atlantic Ocean near Newfoundland was made. Ninety-one strains were recovered from the pelagic and epibenthic regions and tested for 135 binary characters. Using numerical analysis, clusters of related strains were identified and the characteristics of the clusters determined. Pelagic strains were mainly fermentative and clustered separately from the epibenthic strains which were mostly oxidative. Identification was achieved with the aid of type and reference cultures. Fermentative bacteria were tentatively identified as members of the genus Vibrio although some species of Aeromonas were present. Nonfermentative strains were identified as Alteromonas, Flavobacterium and Pseudomonas. Although direct parallels could not be drawn, the bacteria isolated for this region most closely resembled populations described for Alaska, New Zealand and Antarctica.

#### ACKNOWLEDGEMENTS

I wish to thank Dr. John Gow for his advice, encouragement and supervision throughout this project and preparation of this thesis. I thank Dr. Pat Dabinett for his advice, suggestions and comments with respect to the principles of numerical taxonomy, and in the preparation of this thesis. I thank Dr. T. Patel for his suggestions during the initiation and development of this project.

As well, I have greatly appreciated the computer assistance from Ms. Donna Green and Mr. Bill Garland (Computing Services, Medicine) and the cooperation of the Computer Services technical staff during the production of graphical plots and typing of this thesis.

Numerous people have assisted me during the research phase of this project. I thank Mr. Doug Baggs, Ms. Connie Wilson, Mr. Wai Au, Ms. Jennifer Martin and Ms. Janet Nolan. Photographic work was assisted by ETV, Audio-Visual Services (Faculty of Medicine) and Roy Ficken (Biology Dept.). Electron microscopy was done by Ms. Carolyn Emerson.

A special thanks to Ms. Patricia Reddick for her encouragement and help during the final phases of this project.

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## Introduction

### 1. Ecological aspects of marine bacteriology.

Oligotrophic and heterotrophic bacteria are commonly found in the marine environment. Oligotrophic bacteria predominate regions of the ocean that have little or no dissolved carbon and they usually exist in a free-living state regardless of the presence of particulate organic matter. Heterotrophs, on the other hand, reside in areas of high concentrations of dissolved or particulate carbon and are usually in an attached state (Akagi *et al.*, 1977; Fukami *et al.*, 1983).

Investigations into taxonomic representatives of oligotrophic bacteria in the water column are few in number. These bacteria are difficult to isolate and cultivate (Kuznetsov *et al.*, 1979). Numerical taxonomic studies of marine heterotrophs have been more common because these bacteria are readily isolated on a high nutrient source. This group of bacteria readily becomes attached, rapidly metabolizes and reproduces, and is relatively easy to maintain (Horowitz *et al.*, 1983). Oligotrophic bacteria can be isolated on media containing 1 to 15 mg carbon per litre (Kuznetsov *et al.*, 1979) and heterotrophs are usually isolated on media containing 2g or more of carbon per litre (Akagi *et al.*, 1977). Martin and Macleod (1984) have shown,

that both the specific nutrients available and their concentrations can determine which organisms will multiply in a particular environment.

Several numerical taxonomic studies have dealt with heterotrophic bacteria isolated from the marine environment. One of the first numerical taxonomic studies of the heterotrophic bacterial flora of the water column was done by Pfister and Burkholder (1965). Morphological, physiological and biochemical tests showed that classification of organisms was possible using standard computer programs. Of 9 phenetic clusters obtained, 5 clusters were tentatively identified as pseudomonads with different degrees of similarity. Although one cluster was unidentifiable, Achromobacter and Flavobacterium were tentatively identified as well as species from the Family Micrococcaceae.

Quigley and Colwell (1968) investigated bacteria isolated from the water column in the Pacific ocean. Using numerical taxonomy, they observed five distinct phenetic clusters and were able to identify species of the genus Pseudomonas and Aeromonas. Their study showed that bacteria isolated from the deep-sea mud-water interface and those of the sea water were distinctly different.

Singleton and Skerman (1973) compared 155 strains of marine bacteria, from depths of 50 to 2000 m, with 36 luminous bacterial strains from deep-sea fish isolated off

the continental slope of New Zealand. Seven principle clusters resulted from numerical analyses. Cluster I, which consisted of bacteria isolated from 50 and 1000 m, was identified as Moraxella. Clusters II and III were comprised of strains isolated from 50, 500 and 1000 m and were identified as Vibrio. Clusters IV and V, recovered from 50 and 1000 m respectively, were identified as Pseudomonas. Clusters IV and V were identified as Vibrio fischeri and Photobacterium phosphoreum. Their results demonstrated that Photobacterium phosphoreum existed in both the water column and the luminescence organs of deep-sea fish.

Kaneko *et al.* (1979) showed that the dominant bacteria in the Beaufort Sea were different from bacteria isolated in temperate waters. Orange-pigmented Flavobacterium (or Cytophaga) predominated the surface waters in the Beaufort Sea. Using numerical taxonomy they tentatively identified groups of the genus Microcylus, Vibrio, Pseudomonas, Arthrobacter and Acinetobacter.

Austin *et al.* (1979) investigated marine bacteria from two geographically different estuaries. Bacteria from each estuary clustered differently. Strains from Chesapeake Bay were tentatively identified as Acinetobacter-Moraxella, Caulobacter, coryneforms, Pseudomonas and Vibrio while Acinetobacter-Moraxella and Caulobacter predominated the water column samples from Tokyo Bay.

Hauxhurst *et al.* (1980) compared strains from the

Northeastern and Northwestern Gulfs of Alaska. Using numerical taxonomy they tentatively identified strains belonging to the genus Vibrio, Flavobacterium, Bacillus, Pseudomonas and Moraxella-Acinetobacter in the Northeastern Gulf. Vibrio, Flavobacterium, Micrococcus and Chromobacterium were tentatively identified from the Northwestern Gulf of Alaska. Their study showed that most strains tended to cluster with strains of similar regional origin. They attributed this disparity to regional differences in nutrient availability in the water column.

Bacterial flora of the seaweed Alaria esculenta and the giant scallop Placopecten magellanicus isolated near the coast of Newfoundland was investigated by Hollohan (1982). Six major clusters of fermentative strains were identified belonging to the genus Vibrio. Although one cluster of the fermentative strains contained the type strain Vibrio anguillarum biotype II, the other clusters could not be identified beyond the genus level. Of the oxidative strains recovered, Hollohan (1982) described strains that were similar to Pseudomonas marina and Alteromonas haloplanktis on a phenotypic and genetic basis. He described strains of the genus Alteromonas and Pseudomonas which could not be identified beyond the genus level. These unidentified clusters may represent undescribed species.

In summary, the following genera have been identified in the marine environment using numerical taxonomic

analyses: Aeromonas, Vibrio, Photobacterium, Pseudomonas, Achromobacter, Flavobacterium, Cytophaga, Alteromonas, Acinetobacter-Moraxella, Arthrobacter, Caulobacter, Microcyclospus, Bacillus, and Chromobacterium.

## 2. Taxonomic aspects of marine bacteriology.

Currently accepted determinative schemes for the identification of several genera of marine bacteria are based on the work initiated by Baumann *et al.* (1971) and Baumann *et al.* (1972). Baumann *et al.* (1971) investigated fermentative bacteria of marine origin. Based on bacterial tests largely from Stanier *et al.* (1966) and using numerical clustering methods, 6 groups of Vibrio sp., and one group designated as Photobacterium fischeri were described. Due to the ambiguity of the description of the genus Vibrio, they proposed the genus Beneckea to describe those fermentative bacteria which required sodium-ions, were of marine origin and were previously described as Vibrio. Two previously described Vibrio were designated Beneckea alginolytica and B. natriegens while four new species were added to the genus Beneckea, namely; B. campbelli, B. neptuna, B. nereida and B. pelagica. This reassignment of some of the species of Vibrio was upheld (Hugh *et al.*, 1975) but was again re-evaluated (Baumann *et al.*, 1980), and the generic designation reverted to Vibrio instead of the genus

Beneckeia.

In the ninth edition of Bergey's Manual (Krieg, 1984) 20 species and biovars of the genus Vibrio are recognized. All species have a definite sodium-ion requirement and have been isolated from the marine environment.

Since the writing of the ninth edition, several new species have been re-evaluated or described. Vibrio ordalii, has been proposed for V. anguillarum biovar 2. It is distinguishable from V. anguillarum biovar 1 by negative reactions with the Voges-Proskauer test, arginine in Moeller's medium, Simmons' and Christensen's citrate test, ONPG test, amylase, lipase, growth at 37 deg C and failure to ferment cellobiose, glycerol, sorbitol and trehalose (Schiwe *et al.*, 1981).

Love *et al.* (1981) proposed V. damsela for strains that had the ability to form ulcers on the epidermis of the damsel fish. Davis *et al.* (1981) investigated strains of Vibrio isolated from shellfish, water, human diarrheal stools and ear infections. They were able to describe a new species, V. mimicus, which can be differentiated from other strains by its inability to grow on sucrose, lack of the extracellular enzyme lipase, negative reactions with both the Voges-Proskauer test and Jordan tartrate reaction, and sensitivity to polymyxin. Vibrio hollisae, a species not yet recovered from the marine environment, was isolated from human diarrheal stools. This strain may be responsible for

diarrhea in humans who have eaten raw fish (Hickman *et al.*, 1982). *V. diazotrophicus* was described by Guerinot *et al.* (1982). This species is distributed throughout marine and estuarine environment and the gastro-intestinal tracts of marine animals. This species is clearly different from other species of *Vibrio* in it's ability to fix nitrogen by a nitrogenase enzyme. *Vibrio orientalis* was isolated off the coast of China (Yang *et al.*, 1983). Unlike other vibrios, it is unique in its ability to luminesce, accumulate poly- $\beta$ -hydroxybutyrate as an intracellular reserve product and utilize DL- $\beta$ -hydroxybutyrate, putrescine and spermine as carbon sources. Tison and Sneedler (1983) proposed the name *Vibrio aestuarinus* for vibrios having arginine dihydrolase activity, negative Voges-Proskauer test and fermentation of sucrose and lactose. This species was isolated from shellfish in estuarine environments.

Baumann *et al.* (1972) also investigated non-fermentative marine bacteria. Based on a wide variety of morphological, physiological and nutritional characters they were able to identify 22 groups on the basis of phenotypic similarities. Peritrichously flagellated groups with a molecular DNA % G + C values of 53.7 to 67.8 were assigned to the genus *Alcaligenes*. Polar flagellated groups were assigned to either the genus *Alteromonas* (mol DNA % G + C 43.2 to 48.0) or *Pseudomonas* (mol DNA % G + C 57.8 to 64.7). Four species were assigned to the genus *Alteromonas*.

(*A. communis*, *A. vaga*, *A. macleodii* and *A. marinopraesens*), three species to the genus *Pseudomonas* (*P. doudoroffi*, *P. marina*, and *P. nautica*) and four species to the genus *Alcaligenes* (*A. pacificus*, *A. cupidus*, *A. venustus* and *A. aeustus*).

Studies on evolutionary relationships based on ribosomal ribonucleic acid (rRNA) studies revealed that *Alcaligenes aeustus*, *A. pacificus*, *A. cupidus*, *A. venustus* and *Pseudomonas marina* were derived from fluorescent pseudomonads. Consequently Baumann et al. (1983) proposed that these alcaligenes and pseudomonads be placed in a newly created genus *Deleya*. *Deleya spp.* are characterized by the following criteria; accumulation of poly- $\beta$ -hydroxybutyrate (PHB) as an intracellular reserve product, growth at 20 and 35 deg C, and utilization of D-glucose, acetate, propionate, succinate, fumarate, DL- $\beta$ -hydroxybutyrate, DL-lactate, pyruvate, glycerol, L- $\alpha$ -alanine, D- $\alpha$ -alanine, L-glutamate and proline. With the exception of *D. marina*, all species of *Deleya* are peritrichously flagellated.

Studies of rRNA homologies also showed that strain G-1 (ATCC 27130), which previously was not assigned to *Pseudomonas* because of low mol DNA & G + C values, was in fact the same evolutionary linkage as *P. nautica* and *P. doudoroffi*. Therefore this strain was assigned the name *P. stanieri*. Baumann et al. (1983) also studied the

denitrifying marine strain Pseudomonas perfectomarinus.

Based on phenotype, iron-containing superoxide dismutase(Fe-SOD) and glutamine synthetase(GS) sequences he concluded that this species was also part of the same lineage as P. stanieri.

The marine species Pseudomonas nigrificiens produced a black or brown pigment, had a mol DNA % G + C and general phenotypic properties of Alteromonas. On the basis of these characteristics and the similarities observed in GS and Fe-SOD dismutases, this species was designated Alteromonas nigrificiens (Baumann et al., 1984).

Further reassignment of Alteromonas was done when rRNA and DNA hybridization studies excluded A. commis and A. yaga from the genus. Van Landschoot and De Ley (1983) reassigned these species to the genus Marinomonas. Bowditch et al. (1984) showed that A. commis and A. yaga were more related to the genus Oceanospirillum based on Fe-SOD and GS reactions. Groups H-1 and I-1, originally described in Baumann et al. (1972), were assigned as O. kriegii and O. jannaschii respectively.

Recent determinative schemes are available for other genera of aquatic bacteria such as Flavobacterium, Cytophaga, Alteromonas, Moraxella, Acinetobacter and Aeromonas (Bergey's Manual of Systematic Bacteriology, 1984). None of the strains in these genera require sodium-ions for growth and so they can be differentiated

from marine pseudomonads, alteromonads and vibrios on this basis. Flavobacterium strains are aerobic, non-motile, usually orange pigmented and have a mol DNA & G + C of 31 to 42. They can be differentiated from Cytophaga which shows gliding motility (Holmes *et al.*, 1984). Moraxella and Acinetobacter belong to the family Neisseriaceae. Their growth temperature optima are between 32° to 36 deg C, although there are some psychrophilic strains of Acinetobacter (Bovre, 1984). Most strains are parasitic in warm blooded hosts, again with the exception of some strains of Acinetobacter. Acinetobacter is strictly aerobic. Aeromonas and Vibrio both belong to the family Vibrionaceae (Baumann and Schubert, 1984). The primary criteria by which these two genera can be differentiated are mol DNA & G + C and sodium-ion requirement. Vibrio has a mol DNA & G + C of 38 to 51, while Aeromonas has a mole & G + C of 57 to 63. Vibrio also requires sodium-ions for growth, while Aeromonas does not.

### 3. Theoretical aspects of numerical taxonomy.

Numerical taxonomy was defined by Sneath and Sokal (1973) as "the grouping by numerical methods of taxonomic units into taxa on the basis of their character states" and is based on the principles proposed by Michel Adanson. The principles of numerical taxonomy are as follows:

- i) Maximum information content should be obtained for all taxonomic units (strains).
- ii) All characterization tests should have equal weight.
- iii) Taxa should be defined on the basis of overall similarity according to the results of the analyses (Colwell and Austin, 1981).

Similarity among all possible pairs of strains in the data matrix can be determined using a simple matching coefficient or similarity ratio. The difference between these measures is best explained when referring to a 2 X 2 frequency table:

		OTU Y	
		+	-
OTU X	+	A	B
	-	C	D

The letter A refers to the number of tests which scored positive in both OTU and the letter D refers to the number of tests which scored negative in both OTU. The letter B refers to the number of tests which scored positive for OTU X but negative for OTU Y. The letter C, on the other hand, refers to the number of tests which scored negative for OTU X but positive for OTU Y. The sum of A, B, C and D equals the number of binary tests used in the study. Simple matching is expressed as follows:

$$S = A + D / A + B + C + D$$

whereas the similarity ratio (or Jaccard coefficient) is expressed as:

$$S = A / A + B + C$$

Simple matching and Jaccard coefficient differ from one another in that the former takes into consideration both positive and negative matches, while the latter ignores negative matches (Wishart, 1978).

Dissimilarity is measured by comparing the number of tests present in OTU X but absent in OTU Y (B), with the number of tests present in OTU Y that are absent in OTU X (C), over the number of tests (Wishart, 1978). Dissimilarity is a distance measure and may be computed in several different ways. Euclidean distance is used in this study and is expressed as:

$$d(X, Y) = B + C / A + B + C + D$$

Ball, G.H. 1966. A comparison of some cluster seeking techniques. Stanford Res. Inst. Calif.

where  $d$  is the distance between OTU X and Y. This measure is based on the Pythagorean geometry and therefore conforms to the following conditions:

i)  $d(X, Y) > 0$ ;  $d(X, Y) = 0$  then  $X = Y$

ii)  $d(X, Y) = d(Y, X)$

iii)  $d(X, Z) + d(Y, Z) > d(X, Y)$

where Z refers to OTU z (Sneath and Sokal, 1973).

Once measures of similarity or dissimilarity are determined, strains with 'like' qualities can be grouped together using clustering techniques. The object of cluster analyses is to reorganize OTU with similar characteristics so that they have a high degree of association with one another, while OTU with dissimilar characteristics have a low degree of association. Clustering of OTU can be done in a number of ways and is dependent on the similarity or dissimilarity value used. Each technique defines distance or dissimilarity between OTU and groups of OTU in a different way.

Hierarchical clustering is most commonly used in numerical taxonomy and consists of two methods; agglomerative and divisive. Agglomerative techniques perform a series of successive fusions of N entities into groups. That is, this method reduces data to a single cluster containing all entities. Divisive methods

partitions the entire set of data into N groups each containing a single entity (Everitt, 1980).

In this study several agglomerative methods were used, namely: single linkage, average linkage and Ward's method.

Single linkage or nearest neighbour can be used for both similarity and dissimilarity measures. Groups initially consisting of single individuals are fused according to the distance or similarity between nearest members. Groups with the smallest distance or highest similarity are fused and as a consequence, this decreases the number of existing groups.

The disadvantage of this method is the tendency to chain OTU together rather than initiate new clusters (Everitt, 1980).

Average linkage defines the distance or similarity among groups as the average of the distances between all pairs of OTU in the group and fuses those having similar values. A method described by Ward's (1963) utilizes Euclidean distance values and clusters OTU so that the nucleus of each cluster represents an OTU common to all other strains within the cluster. At each step of the analysis, union of every possible pair of clusters is considered and the two clusters whose fusion results in the minimum increase in the errors sum of squares are combined.

Relocate clustering is defined by Everitt (1980), as a divisive form of hierachial clustering. Initially all strains are assigned to one of the N clusters (depending on the order in the data matrix). Each strain is then compared

to other strains within its assigned cluster. If a strain becomes more similar to another cluster during a relocate scan, the strain is transferred and an iteration takes place. That is, the entire cluster is re-evaluated until all clusters become stable (Wishart, 1980).

Density search clustering is used with similarity values. This method calculates the density of space in the immediate vicinity of each individual. Small density values represent regions of high density and vice versa. Individuals are sorted so that the least dense values become the first cluster nuclei. During each cycle the radius of the cluster surrounding the nucleus is increased to the next density value. If the new density value exceeds the radius distance, a new cluster is initiated. However, if the value is within the radius distance, the strain joins the cluster. If the strain is within the radius distance of several clusters, all clusters join to become one (Wishart, 1980).

Most cluster analyses can be represented in the form of two dimensional diagrams which illustrate the fusion or division which has occurred during various stages of the analysis. These are known as dendograms (Everitt, 1980). Computerized output showing characteristics of the clusters at different levels of similarity or dissimilarity also assist in the interpretation of dendograms. Ideally the investigator should designate groups of OTU as clusters at a similarity or dissimilarity level which best separates

groups of OTU on the basis of unique characteristics possessed by groups. This similarity or dissimilarity value is made at the discretion of the investigator.

Several clustering analyses should be performed in order to access the robustness of the clusters. It is an indication of a robust classification if a similar classification scheme is achieved using several different methods (Wishart, 1978). OTU which do not group with similar OTU using different numerical analyses should be regarded as outliers or vagrant OTU. In general, these OTU are members of a group which is poorly represented in the data matrix.

Vagrant OTU may also be an indication of incorrect or insufficient characterization (Everitt, 1980).

#### 4. Objectives of the present study

The focus of this study was to characterize bacteria from the water of the Northwest Atlantic Ocean near Newfoundland and determine characteristics of representative groups using numerical taxonomic techniques. It was of interest to determine similarities and differences between pelagic bacteria isolated from the open water and epibenthic bacteria isolated from the sediment. It was also of interest to compare key representative strains from the study of Hollohan (1982), to determine if pelagic and epibenthic strains were similar to those isolated previously.

from the seaweed Alaria esculenta and the giant scallop  
Placopecten magellanicus.

### Materials and Methods

#### 1. Media used to isolate and maintain the strains.

Natural seawater and artificial seawater media were used in this study. Also, complex and defined media were prepared.

The bacteria were isolated using a complex natural seawater medium described by Gow and Mills (1984). It contained yeast-extract (1% wt/vol), proteose peptone no. 3 (Difco) (1% wt/vol) and 75% natural aged seawater. The medium was solidified with Oxoid technical grade agar no. 3 added at 1.2% (wt/vol). This medium was called YEPN medium.

Some media were prepared with buffered artificial seawater (BASW). The BASW was prepared by mixing equal volumes of 2 components that were prepared at double strength. The two components were an artificial seawater (ASW) and a buffered salts solution (BSS). The single strength composition of ASW was 300mM NaCl, 50mM  $MgSO_4 \cdot 7H_2O$ , 10mM KCl and 1.0mM  $CaCl_2 \cdot 2H_2O$ . The single strength composition of BSS was 50mM Tris(hydroxymethyl)methylamine (Tris)-hydrochloride (ph 7.5), 1.0mM  $(NH_4)_2HPO_4$  and 26 M  $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ . The BASW was devised from formulae by Baumann *et al.* (1971) and Gow *et al.* (1973).

A yeast-extract, proteose peptone medium similar to YEPN was prepared by substituting BASW for the 75% natural

seawater. This medium was designated YEPA.

Luminescence medium (LM) was used to maintain some of the cultures. It consisted of 5% (wt/vol) yeast-extract, 5% (wt/vol) Bacto-Tryptone (Difco), 0.3% (vol/vol) glycerol, 0.1% (wt/vol) CaCO<sub>3</sub> and 1.2% technical grade agar in BASW. This medium was similar to one described by Baumann and Baumann, 1981).

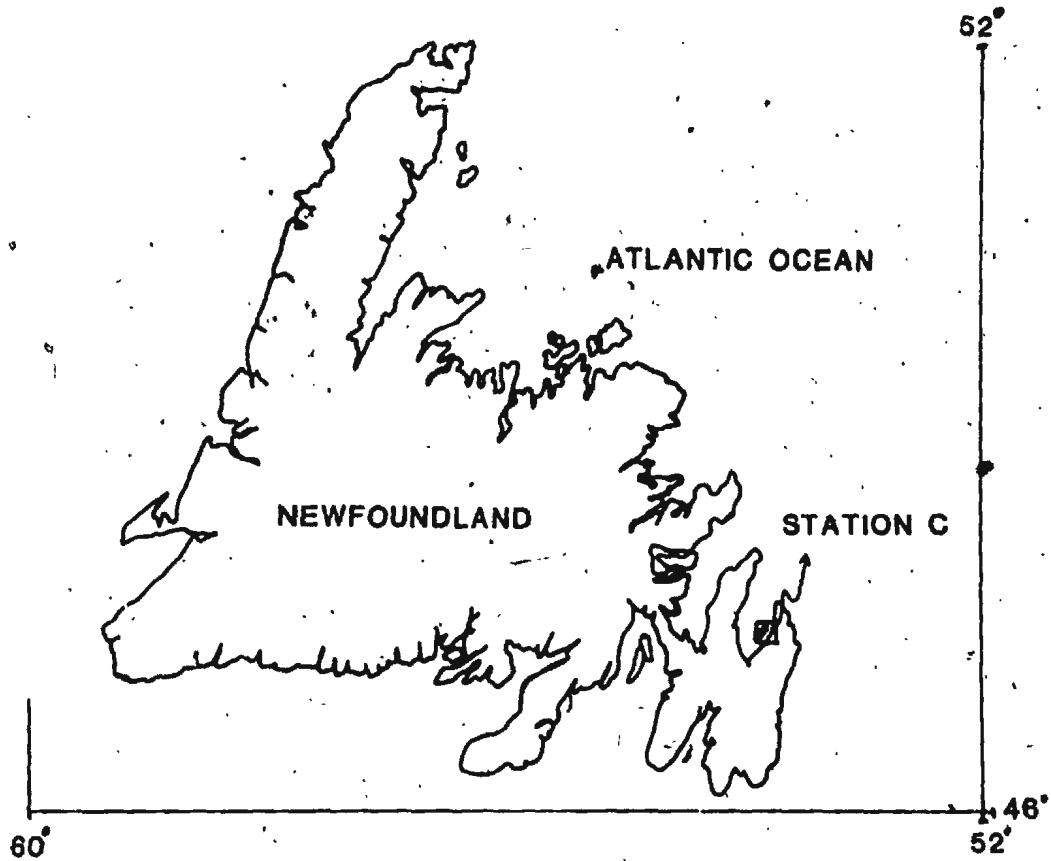
Another medium that was used to maintain cultures was a modified Lib X medium (MLX) (Griffiths et al., 1974). This medium consisted of 1% (wt/vol) Trypticase (BBL), 1% (wt/vol) yeast-extract, 0.03% (wt/vol) sodium citrate, 0.03% (wt/vol) sodium glutamate, 0.005% (wt/vol) NaNO<sub>3</sub> and 0.001% Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O. These ingredients were added to aged 75% (vol/vol) natural seawater buffered with 50mM Tris (pH 7.5). This medium was solidified with 1.2% technical grade agar.

All of the above media were sterilized by autoclaving at 121 deg C for 15 minutes.

## 2. Isolation of the strains.

The first isolation was made from Conception Bay, Newfoundland, Canada (latitude 47°34.4'N, longitude 53°1.5'W) in November, 1982. Samples were taken in the vicinity of a station (Fig. 1) named station C (Powell, 1980). The station was near the east side of Kelly's island

Figure 1. Site of first and second isolation taken  
in November, 1982 and June, 1983. Shown  
is the province of Newfoundland, Canada,  
and the approximate location of Station C  
in Conception Bay.



in a region with sediment on the seabed. Samples were taken from open water (pelagic) and from the surface of the sediment (epibenthic). A second isolation from the pelagic zone was made in June, 1983.

One litre water-samples were collected aseptically at 20 and 60 m. A Johnson-Zobell (J-Z) water sampler (Zobell, 1941) was used to collect pelagic samples from the water column. A 250 ml core sample of sediment was taken at a depth of approximately 61 m. A K.B. design core sampler (WILDCO INSTRUMENTS, Michigan) fitted with a sterile plastic core tube was used to collect sediment from which an epibenthic sample was obtained.

Once the samples were collected they were stored in seawater and ice until they were processed in the laboratory. The time that elapsed between collection and plating of the samples was less than 6 hr. For pelagic samples, individual colonies were obtained by the spin-plate technique. A recent description of this technique was given by Gow and Mills (1984). Ten plates of YEPN medium per sample were inoculated. The epibenthic bacteria were obtained from sediment contained in the sterile plastic cylinder of the core sampler. The top centimeter of sediment was removed aseptically and 1 g wet weight was added to a 99ml dilution blank. This was serially diluted (A.P.H.A., 1976) and YEPN medium was inoculated by the spin-plate technique. The dilution blanks contained 75%

natural seawater.

To obtain the dry weight of the sediment, subsamples were dried at 105 deg C in tared weighing vessels until constant weight was achieved.

All plates were incubated at 15 deg C and observed for growth at two week intervals for a total of 6 weeks.

Colonies with a diameter of 1mm or greater and derived from the epibenthic region were numbered and representative strains were picked by using a table of random numbers.

Similarity colonies derived from the pelagic regions had a diameter of 1mm or greater when picked. The colonies were subcultured onto MLX medium and incubated for 2 weeks at 15 deg C. Cultures were checked for purity by streak-plating and then were routinely transferred on either MLX medium or LM medium. Sets of stock cultures were maintained on YEPA slants. After good growth was obtained tubes were filled with sterile mineral oil until the sloped portion was covered. This increased the storage life of the stock cultures.

### 3. Binary characterization tests..

Most characterization tests were done following procedures described in Hollohan (1982). Those tests which were essentially unchanged are mentioned briefly, but tests which were revised or modified are mentioned in greater

detail. Procedures for many of the tests can also be found in Colwell and Wiebe (1970), Baumann and Baumann (1981) and the Manual of Methods of General Bacteriology (Gerhardt, 1981). For those tests that required incubation, 20 deg C was used unless otherwise specified.

a) Gram reaction. The gram reaction was determined using the method suggested by Skerman (1967) and verified by the non-staining KOH technique (Buck, 1982).

b) Motility. Twenty-four to forty-eight hour LM broth cultures were examined by phase contrast optical microscopy. Motility was considered positive only if undisputable movement was observed (Colwell and Wiebe, 1970).

c) Leifson MOF (oxidation-fermentation) test. Bacto-MFO medium supplemented with 1.0% membrane sterilized glucose was used to differentiate oxidative and fermentative bacteria. Fermentation was determined using MFO medium overlaid with sterile mineral oil. Fermentative bacteria produced acid in the anaerobic test tubes while oxidative bacteria produced acid only in the aerobic test tubes. Bacteria which did not produce acid in either test tube were considered MFO negative (Leifson, 1963).

d) Oxidase test. Several drops of freshly prepared tetramethyl-paraphenyl-di-aminedihydrochloride in 0.2% ascorbic acid were added to a sheet of Whatman's filter paper. A thick inoculum was obtained using a sterile cotton swab, and dabbed on the soaked filter paper. The

development of a blue-purple colour within 60 seconds indicated a positive test (Chruckshank et al., 1975).

e) Arginine dihydrolase. BASW lacking TRIS-HCl (ph 7.5) was prepared with the addition of 1.0% (wt/vol) L-arginine, 0.1% (wt/vol) proteose peptone No. 3, 10 mg/l phenol red and 0.2% (wt/vol) technical agar. A control medium was also prepared which consisted of all the above with the exception of L-arginine. The tubes were incubated and overlaid with sterile mineral oil. Cultures were inoculated four to seven days. A positive reaction was noted by a difference in colour due to an increase in alkalinity in the L-arginine tubes (Baumann and Baumann, 1981).

f) Denitrification. Denitrification medium consisted of BASW supplemented with an additional 0.6% (wt/vol) TRIS (pH 7.5), 0.5% (wt/vol) yeast extract (Difco), 0.1% (wt/vol) sodium succinate, 0.1% (wt/vol) sodium acetate, 0.1% (wt/vol) sodium lactate, 0.2% (wt/vol) technical agar No.3 and 0.3% (wt/vol)  $\text{NaNO}_3$ . The cultures were first inoculated in denitrification medium and incubated for 48 to 72 hours. Then they were transferred to new denitrification medium and overlaid with mineral oil. Growth was observed for 2 weeks. A test was considered positive if gas was produced (Baumann and Baumann, 1981).

g) Sodium-ion requirement. Sodium-ion requirement was determined using sodium-free BASW supplemented with 1.0% (wt/vol) glucose and 1.0% (wt/vol) glutamic acid in free

acid form. This medium was adjusted to pH 7.5 with NH<sub>3</sub>OH instead of NaOH. Cultures were first suspended in BASW lacking NaCl and then resuspended in sodium-free medium containing 1% (wt/vol) Oxoid purified agar. A similar medium but containing sodium-ions, was inoculated as a control. Both sodium and sodium-free media were autoclaved and cooled before the addition of membrane-sterilized galactose solution. After inoculation, sodium and sodium-free media were incubated for 1 week and observed for the presence or absence of growth.

h) Growth temperatures. Strains were suspended in test tubes containing YEPA broth and incubated in circulating water-baths at 5, 35 and 40 deg C. Any visible growth observed in three weeks was considered positive. Controls were incubated at 20 deg C to ensure viability of the strains during the test period.

i) Nutritional screening. The following compounds were tested as sole sources of carbon: (alcohols) ethanol, isopropanol, n-butanol, propanol, erythritol, D-mannitol, adonitol, D-sorbitol, meso-inositol, glycerol; (amino acids) glycine, L-alanine, D-alanine, D-alanine, L-serine, L-theanine, L-leucine, L-isoleucine, DL-norleucine, valine, ornithine, DL-aspartate, L-lysine, L-arginine, DL- $\alpha$ -amino-butyrate,  $\alpha$ -aminovalerate,  $\beta$ -aminovalerate, n-acetylglucosamine, L-glutamate, DL-citrulline; (carbohydrates) D-ribose, L-arabinose, D-xylose,

D-trehalose, D-cellulose, D-fucose, D-galactose, D-glucose, D-melibiose, cellulose, D-fructose, D-mannose, lactose, maltose, sucrose, L-rhamnose, salicin, D-glucurinate, D-galacturonate, gluconate, mucate, inulin, D-saccharate; (carboxylic acids) acetate, isobutyrate, propionate, butyrate, isovalerate, pelargonate, heptanoate, glycocholate,  $\gamma$ -amino butyrate, valerate, oxalate, malonate, formate, pimelate, suberate, succinate, adipate, azelate, sebacate, maleate, fumerate, DL-malate, L-malate, DL-glycerate, DL-lactate, DL- $\alpha$ -hydroxybutyrate, DL-tartrate, L-tartrate, meso-tartrate, glycolate, citrate,  $\alpha$ -ketoglutarate, pyruvate, itaconate, aconitate; (other amino acids and related compounds) L-histidine, L-proline, L-tyrosine, kynurenate, L-phenylalanine, L-tryptophan, p-aminobenzoate, putrescine, DL-kynurenate, ethanolamine, D-tryptophan, benzylamine; (non-nitrogenous aromatic and other cyclic compounds) D-mandelate, L-mandelate, benzoate, m-hydrobenzoate, phthalate, quinate, p-hydrobenzoate; (other nitrogenous compounds) betaine, sarcosine, creatine, hippurate, niacinamide, nicotinate, allantoin and adenine.

The carbon sources were sterilized either by autoclaving or by membrane filtration. The appropriate method for each compound was given by Hollohan (1982) and Palleroni and Doudoroff (1972). Carbohydrates were prepared at 0.2% (wt/vol) in BASW with 1% Oxoid purified agar.

Carbon sources were tested at 0.1% w/v in BASW with 1%

purified agar. Acidic or basic carbon sources were adjusted to pH 7.5. Strains were cultured for 24 to 48h on LM agar prior to screening to ensure good growth. Strains were then added to BASW until a light turbidity was detected. These suspensions were then used as inocula. Media were inoculated with a multipoint inoculator (Lovelace and Colwell, 1968) used with a multiwell tissue culture plate (Becton Dickinson and Co., Ca.). The well capacity was 2.8 ml and the media to be inoculated had been poured into 100 X 100 X 15 mm square petri dishes. A control consisting of MLX agar ensured viability of the strains. Another control consisted of BASW agar without a carbon source. This latter control was used to determine the amount of growth that occurred in the absence of nutrients.

j) Voges-Proskauer (VP) test. VP test was done by the method of Blazevic and Ederer (1975) using BASW instead of distilled water to prepare the medium.

k) Fluorescence. Fluorescence activity was observed by growing the strain in MLX medium. The cultures were examined in normal day-light for the production of fluorescence.

l) Extracellular enzymes. All exoenzyme tests were conducted using the multipoint inoculum method (Lovelace and Colwell, 1968). Amylase, gelatinase, lipase and cellulase activity were determined using YEPA medium supplemented with 0.2% (wt/vol) starch, 2.0% (wt/vol) gelatin, 1.0% (vol/vol)

tween, 80 or 1.0% (wt/vol) sodium carboxymethyl-cellulose respectively. Alginase testing was done by overlying YEPA agar with YEPA AGAR plus 2.0% (wt/vol) sodium alginate. Laminarinase was prepared by spreading a thin layer of 4% (wt/vol) laminarin (Sigma) over a base of YEPA agar. Chitin medium was prepared by overlaying YEPA agar with YEPA agar supplemented with an additional 0.25% (wt/vol) yeast extract and 0.5% (wt/vol) colloidal chitin.

After 48 hours the starch plates were flooded with Lugol's iodine solution. A positive amylase test was shown by the production of a clear zone surrounding the inoculum point. Gelatinase and cellulase were also tested after 48 hours. Gelatin plates were flooded with 30% trichloroacetic acid. A zone of clearing indicated positive gelatinase activity. Cellulose plates were first flooded with 1 mg/ml Congo red in distilled water (15 min), followed by 1M. NaCl (15 min). A clear zone indicated cellulase activity. Alginase, chitinase, and laminarinase activities were noted by a clear zone surrounding the inoculum point. Agarase activity was noted by a prominent depression in the agar. Most of the above procedures have been described by Baumann and Baumann (1981). The test for cellulase was described by Teather and Wood (1982).

4. Flagella staining.

Flagella staining was best achieved using the Leifson staining technique (Gerhardt, 1981). Cells were slowly shaken in MLX broth for 24-72 hours. Then 2 to 5 ml of inoculated broth were added to 10% (vol/vol) aqueous formalin until a faint turbidity was detected. Several ml of this suspension were very carefully poured over an acid-cleaned slide tilted at approximately a 45 deg angle. After air drying, the slide was marked around the borders with a wax pencil. Approximately 1 ml of dye solution was added for 7 to 10 minutes and then floated off with tap water. The flagella and cells, which stained red, were examined by light microscopy.

5. Poly- $\beta$ -hydroxybutyric acid accumulation.

Strains were grown on medium consisting of BASW with 0.02% (wt/vol)  $(\text{NH}_4)_2\text{SO}_4$  and 0.04% (wt/vol) DL- $\beta$ -hydroxybutyric acid for 48 to 72 hours. The isolation and estimation of PHB was done by the method described by Slepecky and Law (1960). A 2-ml portion of each culture was added to 8 ml of 5% hypochlorite (bleach). After 24 hours, the strains were centrifuged (10,000 X g) for approximately 20 minutes. The supernatant was carefully discarded and the cells were purified by a series of suspensions and centrifugations using distilled water, acetone (2 times),

and diethyl ether (2 times). After drying for 24 h, the cells were dissolved in 2 ml H<sub>2</sub>SO<sub>4</sub> while heated in boiling water for 10 min. A positive test for poly-β-hydroxybutyric acid accumulation was determined by observing an absorption peak at 235 nm due to the presence of crotonic acid.

#### 6. Aromatic ring cleavage.

Strains were grown in 250-ml media consisting of either 0.1% (wt/vol) p-hydroxybenzoate or, 0.1% (wt/vol) quinate or 0.15% (wt/vol) sodium benzoate for 48 to 72 hours. Bacterial cells were harvested by centrifugation (15,000 X g for 10 minutes). A suspension was made in 2 ml of 0.2M Tris buffer (pH 8.0) and the cells were gently shaken. Then 0.5 ml toluene and 3.5 mg of protocatechoic acid were carefully added while the cells were lightly shaken at 30 deg C. The production of a yellow colour within several minutes was a positive test for meta cleavage. Excessively hard shaking could have resulted in a negative meta cleavage result. If a yellow colour was not apparent, the suspension was lightly shaken for 1 h and checked again. One gram (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 1 drop of sodium nitroferricyanide was added, followed by 0.5 ml ammonia solution (28-30%). The production of a vivid purple colour was considered positive for ortho cleavage (Gerhardt, 1981).

7. Numerical taxonomy.

Each strain was regarded as an operational taxonomic Unit (OTU). Eight replicates were used as controls during the collection of binary data. This was done to ensure good agreement of characterization tests as suggested by Sneath and Johnson (1972). Numerical classification was done by the recommended procedure by Wishart (1978) using the CLUSTAN analysis package implemented on Digital Equipment Corp. Vax 11/780 computer. A binary data set was created. Binary data was coded '0' for negative characters and '1' for positive characters. Euclidean distance was computed and Ward's clustering method was used to group up to 10 clusters. Results of these analyses were presented in the form of dendograms. Relocate clustering was computed using several random clusters. Simple matching coefficient was used with density clustering to give estimates of modes in the sample. Jaccard coefficient with average linkage clustering was also used.

Once clusters were determined, characteristics of the clusters were investigated. Binary frequency scores, which represent the frequency of a test in the cluster over the frequency of the test in all other OTU, were multiplied by the product of the frequency of the test in the cluster over the number of OTU in the cluster. This value, known as the scaled score, was divided by the maximum value of the test

assuming it was ideally 100% unique to the cluster. This computation gave the scaled score of the maximum value which was considered unique if a value was greater or equal to 20%. In this study, scaled score of the maximum value is a simplified discriminant value that identifies test(s) which are unique to a cluster.

#### 8. Type cultures and reference strains.

A list of type cultures and reference strains is given in Table 1. Type cultures were obtained from the American Type Culture Collection, Rockville, Maryland. The appropriate American Type Culture Collection (ATCC) numbers are given. To aid in the identification of pelagic and epibenthic strains, 32 additional type strains were included (Table 2). Some reference strains described by Hollohan (1982) were also used in this study. The generic designation and the number assigned to the strain by Hollohan (1982) is given in Table 3. These cultures were used either in numerical analyses or as positive and negative controls in characterization tests.

#### 9. Electron microscopy.

Representative strains were suspended in MLX broth and lightly shaken for 8 to 12 hours. Approximately 10 ml

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

Type culture	ATCC#
<u>Alteromonas undina</u>	29660
<u>Alteromonas espejiana</u>	29659
<u>Vibrio vulnificus</u>	27562
<u>Vibrio splendidus</u>	25914
<u>Alteromonas macleodi</u>	27126
<u>Photobacterium angustum</u>	25915
<u>Alcaligenes aquamarinus</u>	14400
<u>Deleya venusta</u>	27125
<u>Pseudomonas nautica</u>	27132
<u>Photobacterium phosphoreum</u>	11040
<u>Oceanospirillum commune</u>	27118
<u>Oceanospirillum vagum</u>	27119
<u>Deleya cupida</u>	27124
<u>Pseudomonas doudoroffii</u>	27123
<u>Pseudomonas fluorescens</u>	E13043
<u>Deleya marina</u>	25374

Table 2: Type and reference cultures used to identify OTU.  
The American Type Culture Collection (ATCC)  
number or Laboratory Centre for Disease Control  
(LDC) number, species, and Operational  
Taxonomic Unit (OTU) designation are given.

ATCC Number	Name	OTU
7744	<u>Vibrio fischeri</u>	7744
E14048	<u>Vibrio natriegenes</u>	E14048
17749	<u>Vibrio alginolyticus</u>	17749
17802	<u>Vibrio parahemolyticus</u>	17802
E19264	<u>Vibrio anguillarum</u>	E19264
25916	<u>Vibrio pelagius</u>	25916
25917	<u>Vibrio nereis</u>	25919
25919	<u>Vibrio harveyi</u>	25919
25920	<u>Vibrio campbellii</u>	25920
27043	<u>Vibrio nigrapulchritudo</u>	27043
33125	<u>Vibrio proteolyticus</u>	33125
33466	<u>Vibrio diazotrophicus</u>	33466
29570	<u>Vibrio gazogenes</u>	29570
14343	<u>Alteromonas haloplanktis</u>	14343
27135	<u>Oceanospirillum jannaschii</u>	27135
27120	<u>Group B-1 Baumann</u>	27120
27128	<u>Alteromonas aestus</u>	27128
8017	<u>Alteromonas putrefaciens</u>	8017
33492	<u>Alteromonas luteoviolacea</u>	33492
29988	<u>Alteromonas rubra</u>	29988
27130	<u>Pseudomonas stanieri</u>	27130
27133	<u>Oceanospirillum kriegii</u>	27133
27121	<u>Group B-2 Baumann</u>	27121
29985	<u>Vibrio logei</u>	29985

LDC Number	Name	OTU
7588	<u>Vibrio damsela</u>	LDC7588
7588~	<u>Vibrio damsela</u>	LDC2588
75	<u>Vibrio hollisae</u>	LDC75
9012	<u>Vibrio ordallii</u>	LDC9012
9013	<u>Vibrio costicola</u>	LDC9013
9067	<u>Vibrio alonsis</u>	LDC9067
9555	<u>Vibrio fluvialis</u>	LDC9555
9578	<u>Vibrio metschanikovii</u>	LDC9578

\* : number assigned in this study.

~ : replicate.

Table 3: Reference Cultures described by Hollohan (1982) that were included in this study. Shown is the original source of the OTU, MFO reaction and OTU designation used in this study.

Hollohan (1982) number	Source	MFO reaction	OTU~
OTU 44	<u>Alaria esculenta</u>	fermentative	BRN44
OTU 21	<u>Alaria esculenta</u>	fermentative	BRN21
OTU 93	<u>Placopecten magellanicus</u>	fermentative	BRN93
OTU 99	<u>Placopecten magellanicus</u>	fermentative	BRN99
OTU 115	<u>Placopecten magellanicus</u>	fermentative	BRN115
OTU 18	<u>Alaria esculenta</u>	fermentative	BRN18
OTU 117	<u>Placopecten magellanicus</u>	oxidative	BRN117
OTU 121	<u>Placopecten magellanicus</u>	oxidative	BRN121
OTU 67	<u>Alaria esculenta</u>	oxidative	BRN67
OTU 133	<u>Alaria esculenta</u>	oxidative	BRN133

: number assigned in this study.

aliquots of suspension were transferred to test tubes containing 0.1 ml of 37% formaldehyde (adjusted to pH 7.5) and centrifuged at 3,000 X g for 10 minutes. The supernatant was discarded carefully and the cells were suspended in 10 ml of distilled water and then centrifuged two more times. After the last centrifugation, the cells were suspended in 1 ml distilled water.

This suspension was negatively stained with uranyl acetate which were added at a 1:1 ratio. One drop of this mixture was placed on a 200 mesh size carbon Formvar coated copper grid for 1 minute. After drying the grid was examined using a Zeiss EM 9 A electron microscope.

## RESULTS

### 1. Isolation of strains.

The numbers of bacteria present in the pelagic and epibenthic zones of the water column are given in Table 4. Overall bacterial counts were highest in the epibenthic zone and are expressed as colony forming units (CFU) per g dry wt of sediment. Since the number of epibenthic bacteria were numerous, 175 strains were selected for subculturing. However, many of these strains were difficult to maintain and, over a period of several months, there was ca. 73% mortality. In total 36 out of the 175 strains from the epibenthic zone were successfully cultivated. Because the number of colonies per ml seawater from the pelagic zone was low, it was decided to isolate all of the strains from a 1 ml sample from each of two depths. Mortality was again high and only 28 pelagic strains were cultivated successfully from the November sample. Again, the loss of strains on subculture occurred over a period of several months.

Because only 64 strains were successfully cultivated from the sample taken in November, it was decided to obtain additional samples and a second set was collected in June, 1983. The epibenthic zone was not sampled on this occasion. In general, the number of colony forming units per ml was higher during the June isolation than in November. However, the number of strains successfully cultivated was still low. This time a total of 27 strains out of 174 found in 2 ml

Table 4: Number of colony forming units (CFU) in the pelagic and epibenthic zones. The number of strains initially subcultured (S) and the number successfully cultivated (C) are given.

TIME OF SAMPLING	ZONE									
	PELAGIC					EPIBENTHIC				
	20m		60m		61m		CFU/g		S	C
	CFU/ml	S	C	CFU/ml	S	C	CFU/g	S	C	
Nov., 1982 (1st isolation)	37	37	10	~	22	22	18	1804	175	36
June, 1983 (2nd isolation)	100	100	3	74	74	24	-	-	-	-

~ : grams (dry weight).

- : not sampled.

seawater was successfully maintained on subculturing.

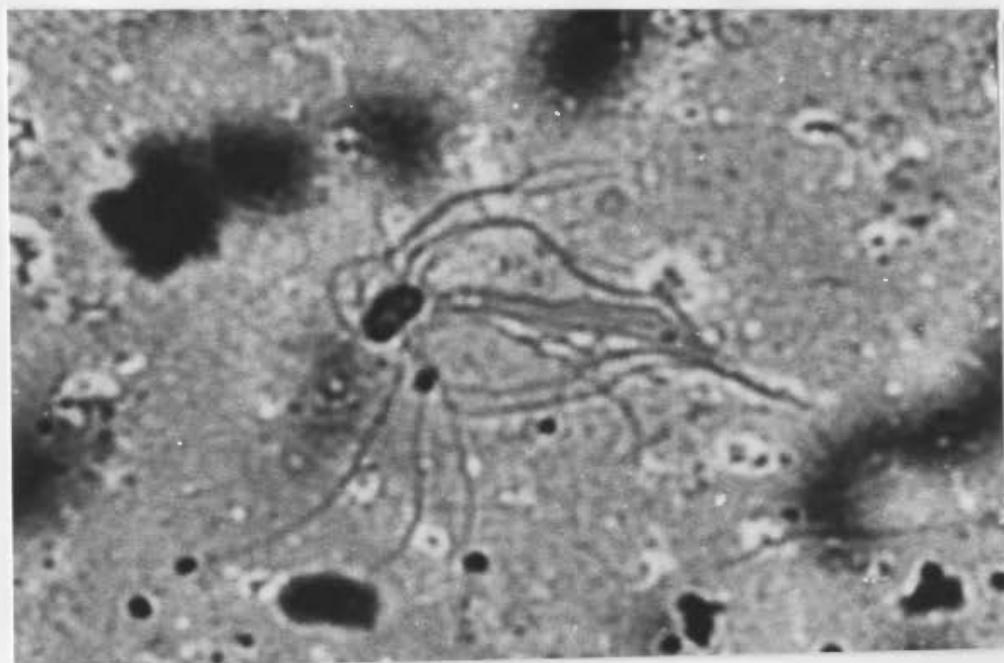
Therefore, the total number of strains or operational taxonomic units (OTU) isolated from the pelagic and epibenthic zones of the water column was 91. Of these, 28 were isolated from the pelagic zone in November, 1982, 36 were isolated from the epibenthic zone in November, 1982 and 27 were isolated from the pelagic zone in June, 1983. Additional type cultures were added to make 116 OTU.

## 2. General characterization of the OTU.

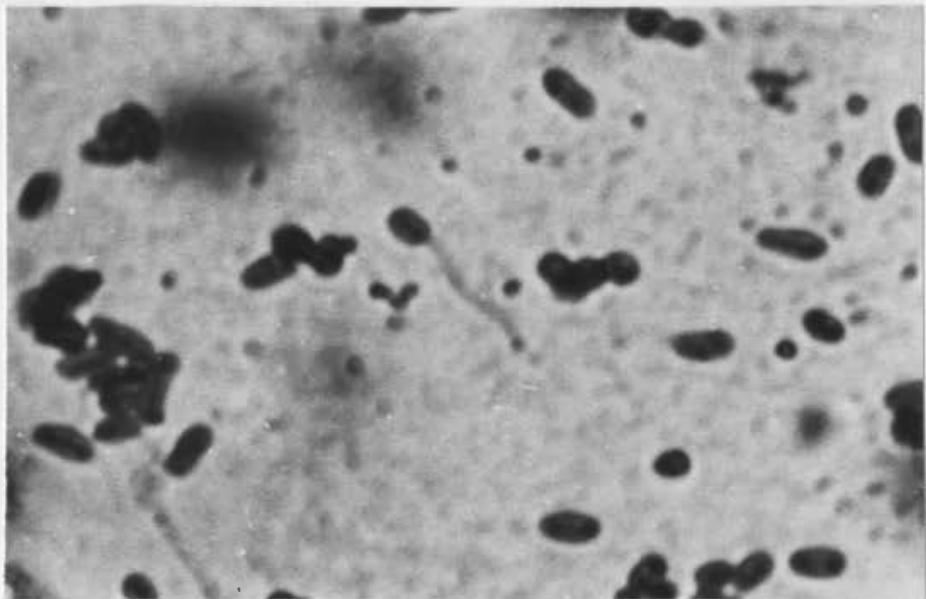
All OTU were gram negative, catalase positive, and unable to produce arginine dehydrolase. No OTU were able to utilize  $\alpha$ -aminovalerate or DL- $\alpha$ -aminobutyrate as sole sources of carbon. With the exception of type and reference strains no OTU were able to accumulate PHB as an intracellular reserve product. All OTU gave a negative Voges-Proskauer reaction.

Not all the OTU were motile but, of those that were, all had polar flagella. Examples of flagellated OTU are given in Figs. 2a, 2b and 3. Fig. 2a shows a peritrichous type of flagellation (*Alcaligenes aquamarinus*), and Fig. 2b, a polarly flagellated organism (OTU 27) from this region. Both were stained by the Leifson's method. An example of a polarly flagellated organism (OTU 100) which was determined by transmission electron microscopy is given in Fig. 3.

Figure 2a. Peritrichous flagellation of type culture  
Alcaligenes aquamarinus stained using  
Leifson's staining technique.



**Figure 2b.** An example of polarly flagellated organism  
(OTU 27) stained by Leifson's staining  
technique.



**Figure 3. Electron micrograph showing OTU 100 with  
two polar flagella. These flagella may be  
distinguished by viewing this photography  
under a stereoscope (12,000 X).**



In order to determine if strains from the pelagic zones and the epibenthic zones would be different based on physiological and biochemical properties, a data matrix was constructed which consisted of 115 OTU and 135 binary characters. The 115 OTU consisted of 91 pelagic and epibenthic strains, 16 type strains and 8 replicates. Replicates were used as a check for assessing accuracy. A list of the 115 OTU is given in Table 1, Appendix A. A list of the 135 binary tests used in the numerical analysis of the 115 OTU is given in Table 2, Appendix A. The results obtained for every OTU scored as binary characters, are given in Table 3, Appendix A. The data were analysed by Euclidean distance and Ward's clustering method the dendrogram shown in Fig. 4. At a dissimilarity value of 0.596, there were ten clusters by Euclidean distance and Ward's clustering method (Fig. 5). A summary of properties common to each cluster is given in Table 5. With the exception of 3 OTU in cluster A, pelagic OTU from the November isolation were found only in clusters B, C and D. OTU from the June pelagic sample grouped in clusters A, I and J with the majority occurring in cluster A. Epibenthic OTU occurred in all clusters with the exception of clusters I, H and J although the majority were found in clusters G and E. Only epibenthic OTU were found in clusters G, E, and F. Overall, the majority of OTU from the pelagic OTU clustered separately as did epibenthic OTU. The majority of

**Figure 4.** Dendrogram obtained using 135 binary tests,  
115 OTU, Euclidean distance coefficient and  
Ward's clustering method. The Y-axis gives the  
dissimilarity at which OTU and clusters of OTU  
merge.



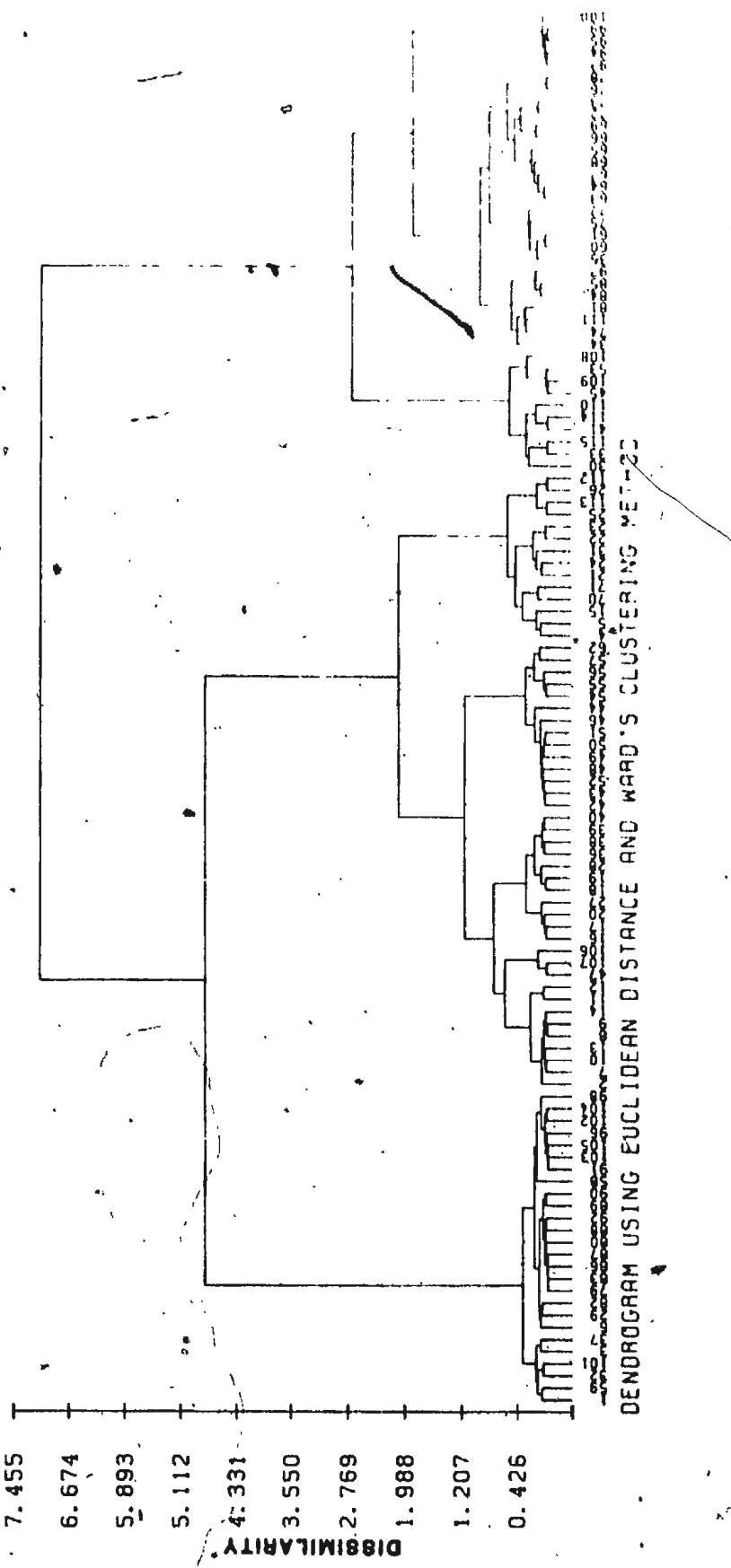


Figure 5. An abbreviated version of a dendrogram obtained using 135 binary tests, 115 OTU, Euclidean distance coefficient and Ward's clustering method. Ten clusters were obtained at a dissimilarity value ca. 0.6. Numbers below cluster lettering indicates number of OTU associated with cluster. The Y-axis gives the dissimilarity values at which OTU and clusters of OTU merge.

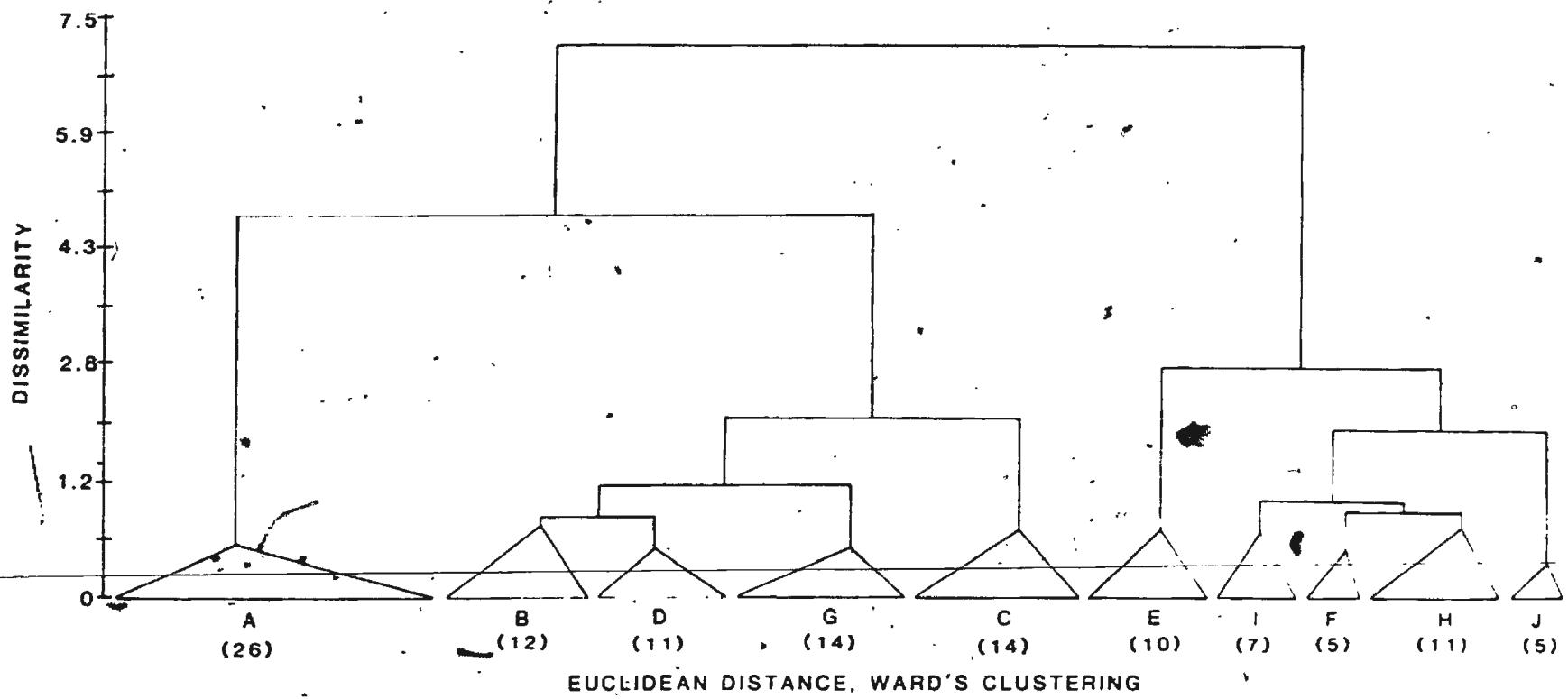


Table 5. Relationship between zone of isolation, Leifson's (oxidation-fermentation) test (MFO), sodium-ion requirement, motility and pigmentation in clusters obtained by Euclidean distance and Ward's clustering method.

ZONE OF ISOLATION AND CHARACTERS	CLUSTER									
	A (26)	B (12)	D (11)	G (14)	C (14)	E (10)	I (7)	F (5)	H (11)	J (5)
<b>ZONE</b>										
PELAGIC (1ST ISOLATION)	3	9	7	-	11	-	-	-	-	-
PELAGIC (2ND ISOLATION)	18	-	-	-	-	-	5	-	-	5
EPIBENTHIC (1ST ISOLATION)	5	3	4	14	1	10	-	3	-	-
TYPE STRAINS	-	-	-	-	2	-	2	2	11	-
<b>CHARACTER</b>										
FERMENTATIVE	16	7	9	-	13	-	5	-	2	5
OXIDATIVE (a)	2	3	1	14	1	5	-	5	8	-
NO REACTION SODIUM-ION REQUIREMENT	8	2	1	-	-	5	2	-	1	-
MOTILITY	16	12	11	11	14	13	2	2	5	9
PIGMENT (NON-SOLUBLE)	21	-	-	-	-	8	1	-	-	5

: number of OTU in cluster.

\* : no. of OTU out of the total in the cluster that were positive.

- : all strains in the cluster were negative.

(a) : strains that did not utilize glucose gave no reaction in the MFO test.

the type OTU also clustered separately. Most were found in cluster H. There were no pelagic or epibenthic OTU in this cluster. Because of the small number of pelagic OTU from the 20 m depth, no attempt was made to differentiate between pelagic OTU from 20 m and 60 m. For each sampling date the OTU from both depths were considered the same in the analysis and the term pelagic was applied to these OTU regardless of the depth from which they were isolated.

Some key characters were examined to determine further information about the clusters. These characters included oxidative or fermentative metabolism as determined by the MFO test. This test can yield several results. Organisms which utilized glucose aerobically and anaerobically had a fermentative metabolism. Those that utilized glucose aerobically only had an oxidative metabolism. Some organisms were oxidative but did not utilize glucose. These organisms grew on MFO medium but did not show a reaction. The 3 types of reaction are shown in Table 5. Also shown are those strains that required sodium-ions, those that were motile and those for which the colonies had non-soluble pigments such as yellow or orange. The latter test character was of interest because it was readily determined at the time of isolation of the OTU.

The following observations were made (Table 5). Most of the clusters had both oxidative and fermentative OTU although one or the other predominated in most clusters.

Fermentative OTU predominated clusters A, D, C, I and J. These were pelagic OTU from the first and second isolations. Most of the epibenthic strains, shown in cluster G, E and F, had oxidative metabolism. One-half of the OTU in cluster E showed no reaction in the MFO test. These were still considered to have oxidative metabolism. Further calculations showed that 80% of the OTU from the pelagic zone were fermentative and 83% of the epibenthic strains were non-fermentative.

Seventy-seven percent of the OTU required sodium-ions for growth. Of the 26 OTU that did not require sodium-ions, 14 were from the epibenthic sample taken in November, 1982 and 12 were from the pelagic zone taken in June, 1983. Cluster E consisted of predominately oxidative OTU that did not require sodium-ions. Most of these were non-motile and pigmented. Cluster I had fermentative OTU that were non-motile and did not require sodium-ions for growth. These were pelagic strains from the second isolation. Two OTU in this cluster were type cultures that required sodium-ions. Cluster J contained 5 OTU of which none required sodium-ions. All were motile and pigmented. Of the 10 OTU that were in cluster A and did not require sodium-ions, one was epibenthic, the rest were pelagic OTU from the second isolation.

Other biochemical, physiological and nutritional characteristics of the clusters are given in Tables 6a and

Table 6a: Biochemical, physiological and nutritional characteristics of clusters A, B, C, D and E based on Euclidean distance and Ward's clustering method.

TEST NAME	CLUSTER				
	A (26)	B (12)	C (14)	D (11)	E (10)
<b>ALCOHOLS</b>					
ETHANOL	2	+	4	+	+
ISOPROPANOL	-	-	-	1	-
N-BUTANOL	1	-	2	-	-
PROPANOL	2	3	7	3	+
ERYTHRITOL	-	3	3	-	+
D-MANNITOL	5	9	+	+	5
ADONITOL	-	1	2	-	+
D-SORBITOL	-	3	10	7	+
MESO-INOSITOL	1	1	2	-	+
GLYCEROL	2	6	7	5	2
<b>AMINO ACIDS</b>					
GLYCINE	-	7	8	-	-
L-ALANINE	-	9	10	+	-
D-ALANINE	-	+	9	+	+
$\alpha$ -ALANINE	-	-	-	-	-
L-SERINE	-	2	5	-	3
L-THEORINE	1	-	9	4	-
L-LEUCINE	1	+	4	+	4
L-ISOLEUCINE	-	-	-	7	-
VALINE	2	-	-	-	-
DL-NORLEUCINE	-	-	-	-	-
L-ORNITHINE	-	-	-	-	-
DL-ASPARTATE	2	+	7	+	+
L-LYSINE	-	1	-	-	5
L-ARGININE	-	+	5	+	-
DL- $\alpha$ -AMINO-BUTYRATE	-	-	-	-	-
$\alpha$ -AMINOVALERATE	-	-	-	-	-
$\delta$ -AMINOVALERATE	-	+	1	+	5

\* : represents the number of OTU in each cluster

+ : 85% or more OTU positive

- : all OTU negative

# : 2 OTU of the 26 OTU have this character

Table 6a continued ...

## CLUSTER

TEST NAME	A (26)	B (12)	C (14)	D (11)	E (10)
<b>(AMINO ACIDS CONTINUED)</b>					
N-ACETYLGLUCOSAMINE	1	1	11 *	6	-
L-GLUTAMATE	2	+	10	+	-
DL-CITRULLINE	-	-	-	-	-
<b>CARBOHYDRATES</b>					
D-RIBOSE	3	+	11	+	+
L-ARABINOSE	-	-	2	-	5
D-XYLOSE	-	-	-	-	7
D-TREHALOSE	7	4	+	+	+
D-CELLOBIOSE	3	5	9	+	+
D-FUCOSE	-	-	-	-	1
D-GALACTOSE	-	-	5	+	5
D-GLUCOSE	10	+	+	+	+
D-MELIBIOSE	-	-	-	-	3
D-FRUCTOSE	8	+	11	+	5
D-MANNOSE	-	-	+	6	1
LACTOSE	-	-	3	-	+
MALTPOSE	1	2	+	+	1
SUCROSE	4	2	7	4	-
L-RHAMNOSE	1	-	-	-	-
SALICIN	1	3	2	-	+
D-GLUCURONATE	-	-	-	-	6
D-GALACTURONATE	-	-	-	-	-
D-GLUCONATE	3	-	5	1	1
MUCATE	-	1	2	-	+
INULIN	-	1	-	-	-
D-SACCHARATE	-	1	2	-	+
<b>CARBOXYLIC ACIDS</b>					
i) FATTY ACIDS					
ACETATE	2	8	7	+	1
ISOBUTYRATE	-	9	1	+	-
PROPIONATE	-	+	6	+	-
BUTYRATE	2	4	2	1	-
ISOVALERATE	-	-	-	+	-
PELARGONATE	1	-	-	-	-
HEPTANOATE	-	± *	6	-	-
GLYCOCHOLATE	-	± *	3	+	2
γ-AMINOBUTYRATE	1	+	1	+	+
VALERATE	1	+	4	+	-

\* : unique character determined by discriminatory analysis

Table 6a continued ...

TEST NAME	CLUSTER				
	A (26)	B (12)	C (14)	D (11)	E (10)
<b>ii) DICARBOXYLIC ACIDS</b>					
OXALATE	-	-	-	-	4
MALONATE	-	-	-	8 *	+
FORMATE	-	-	-	-	2
PIMELATE	-	-	1	4	+
SUBERATE	1	+	4	+	-
SUCCINATE	3	+	+	+	+
ADIPATE	-	-	-	-	-
AZELATE	2	3	-	-	5
SEBACATE	1	1	10	5	4
MELEATE	-	-	-	-	-
FUMERATE	4	+	+	+	6
<b>iii) HYDROXYACIDS</b>					
DL-MALATE	3	9	3	+	5
L-MALATE	5	3	2	2	7
DL-GLYCERATE	1	1	3	-	+
DL-LACTATE	3	+	5	+	6
DL- $\alpha$ -HYDROXYBUTYRATE	3	+	2	+	1
D-(-)-TARTRATE	-	-	-	-	-
L-(+)-TARTRATE	-	-	1	-	1
MESO-TARTRATE	-	-	-	-	3
GLYCOLATE	-	-	1	-	-
CITRATE	5	3	3	+	7
AL-KETOGUTARATE	3	+	11	+	+
PYRUVATE	4	+	+	+	+
ITACONATE	-	-	-	+	*
ACONITATE	2	+	2	+	2
<b>AMINO ACIDS AND RELATED COMPOUNDS</b>					
L-HISTIDINE	-	+	9	+	+
L-PROLINE	1	+	+	+	+
L-TYROSINE	-	3	4	-	+
KYNURENATE	-	-	-	-	*
L-PHENYLALANINE	-	2	2	-	+
L-TRYPTOPHAN	-	-	-	-	-
P-AMINOBENZOATE	-	-	-	-	-

Table 6a continued ...

CLUSTER

TEST NAME	A (26)	B (12)	C (14)	D (11)	E (10)
(AMINO ACIDS AND RELATED COMPOUNDS CONTINUED)					
PUTRESCINE	-	-	2	-	4
DL-KYNURENATE	-	-	-	-	4
ETHANOLAMINE	-	1	-	-	7
D-TRYPTOPHAN	-	-	-	-	-
BENZYLAMINE	-	-	-	-	-
NON-NITROGENOUS AROMATIC AND OTHER CYCLIC COMPOUNDS					
D-MANDELATE	-	1	-	-	+
L-MANDELATE	-	-	-	-	-
BENZOATE	-	-	-	-	-
M-HYDROXYBENZOATE	-	3	-	-	5
PHTHALATE	-	-	-	-	-
QUINATE	-	-	-	-	-
P-HYDROXYBENZOATE	-	-	-	-	-
OTHER NITROGENOUS COMPOUNDS					
BETAIN	-	-	-	-	5
SARCOSINE	-	1	-	-	+
CREATINE	-	-	2	-	+
HIPPURATE	1	2	2	-	-
NIACINAMIDE	1	-	-	-	-
NICOTINATE	-	-	-	-	-
ALLANTOIN	-	-	-	-	-
ADENINE	-	-	-	-	+
ENZYME REACTIONS					
GELATINASE	7	3	+	*	5
AGARASE	-	-	-	-	-
ALGINASE	-	-	-	-	-
CATALASE	+	+	+	+	+
CELLULASE	4	1	+	*	5
CHITINASE	1	9 *	1	-	-
LAMARINASE	1	-	-	-	-
AMYLASE	1	+	10	+	+
LIPASE	2	2	8	4	-
OXIDASE	12	+	+	+	2

Table 6a continued ...

CLUSTER

TEST NAME	A (26)	B (12)	C (14)	D (11)	E (10)

GROWTH TEMPERATURES

GROWTH (5 deg C)	+	+	+	+	+
GROWTH (35 deg C)	+	5	7	4	+
GROWTH (40 deg C)	5	3	2	2	7

OTHER CHARACTERISTICS

DENITRIFICATION  
FLUORESCENCE

6b. Table 6a gives characteristics of cluster A to E and Table 6b gives similar information for clusters F to J.

Cluster A (Table 6a) contained fermentative and oxidative OTU mostly from the epibenthic zone and the pelagic zone, second isolation. These OTU used few of the 112 sources of carbon. None of the 135 binary characters was unique to the cluster.

Cluster B (Table 6a) contained motile, sodium-ion requiring strains from the first isolation and most of these OTU were pelagic. Chitinase activity and the ability to utilize glycocholate and heptanoate as sole sources of carbon were unique to this cluster.

Cluster C (Table 6a) consisted predominately of fermentative OTU from the pelagic region collected in November. This cluster also consisted of one epibenthic OTU and the type cultures: Photobacterium phosphoreum and Alteromonas undina. All OTU required sodium-ions and were motile. Extracellular lipase, gelatinase, cellulase and amylase production were unique to this cluster. In addition, most strains were able to utilize D-mannose, n-acetylglucosamine and maltose as sole carbon sources.

Cluster D (Table 6a) consisted of pelagic and epibenthic OTU from the first isolation. Seven strains were isolated from the pelagic zone and 4 were epibenthic strains. All OTU were motile and required sodium-ions for growth. Only two OTU were oxidative, the others were

Table 6b. Biochemical, physiological and nutritional characteristics of clusters I, F, G, H and J based on Euclidean distance and Ward's clustering method.

	CLUSTER				
TEST NAME	I (7) ~	F (5)	G (14)	H -(11)	J (5)
<b>ALCOHOLS</b>					
ETHANOL	+	+	+	8	+
ISOPROPANOL	1	3	-	-	+
N-BUTANOL	3	+	*	1	3
PROPANOL	5	+	2	7	+
ERYTHRITOL	+	-	-	7	+
D-MANNITOL	+	+	1	+	+
ADONITOL	5	1	-	5	+
D-SORBITOL	+	3	-	+	+
MESO-INOSITOL	+	*	1	-	6
GLYCEROL	2	+	-	+	+
<b>AMINO ACIDS</b>					
GLYCINE	-	+	-	+	+
L-ALANINE	2	+	+	+	+
D-ALANINE	5	+	+	+	+
$\alpha$ -ALANINE	-	-	-	+	*
L-SERINE	4	-	-	+	+
L-THEORINE	2	3	-	7	+
L <sup>L</sup> -LEUCINE	+	+	6	+	+
L-ISOLEUCINE	3	*	-	7	+
VALINE	-	1	-	4	+
DL-NORLEUCINE	1	-	-	1	+
L-ORNITHINE	1	-	1	8	+
DL-ASPARTATE	1	-	10	+	+
L-LYSINE	5	4	-	8	+
L-ARGININE	5	3	+	+	+
DL- $\alpha$ -AMINOBUTYRATE	-	-	-	-	-
$\alpha$ -AMINOVALERATE	-	-	-	-	-
$\xi$ -AMINOVALERATE	4	+	+	+	+

~ : represents the number of OTU in each cluster

+: 85% or more OTU positive

-: all OTU negative

\*: unique character determined by discriminatory analysis

# : 8 OTU of the 11 OTU have this character

Table 6b continued ...

CLUSTER

TEST NAME	I (7)	F (5)	G (14)	H (11)	J (5)
<b>(AMINO ACIDS CONTINUED)</b>					
N-ACETYLGLUCOSAMINE	4	1	-	8	+
L-GLUTAMATE	+	+	+	+	+
DL-CITRULLINE	1	-	-	6 *	+
<b>CARBOHYDRATES</b>					
D-RIBOSE	5	3	-	+	+
L-ARABINOSE	3	3	-	8	+
D-XYLOSE	4	-	-	6	+
D-TREHALOSE	5	2	-	8	+
D-CELLOBIOSE	4	2	-	7	+
D-FUCOSE	1	-	-	-	+
D-GALACTOSE	5	3	8	8	+
D-GLUCOSE	+	+	1	+	+
D-MELIBIOSE	5	1	-	7	+
D-FRUCTOSE	+	2	1	+	+
D-MANNOSE	2	1	1	7	+
LACTOSE	-	-	-	3	+
MALTOSE	3	+	-	7	+
SUCROSE	3	2	-	8	+
L-RHAMNOSE	1	1	-	-	+
SALICIN	4	1	-	4	+
D-GLUCURONATE	5	+	-	8	+
D-GALACTURONATE	3	+	-	+	*
D-GLUCONATE	5	+	-	+	+
MUCATE	+	+	-	8	+
INULIN	1	-	-	-	+
D-SACCHARATE	3	+	1	8	+
<b>CARBOXYLIC ACIDS</b>					
1) FATTY ACIDS					
ACETATE	1	+	+	+	+
ISOBUTYRATE	4	+	10	+	+
PROPIONATE	3	+	+	+	+
BUTYRATE	5	+	6	6	+
ISOVALERATE	+	+	6	+	+
PELARGONATE	-	+	1	+	*
HEPTANOATE	5	+	3	+	+
GLYCOCHOLATE	5	3	-	3	+
-AMINOBUTYRATE	5	+	+	+	+
VALERATE	4	+	+	+	+

Table 6b continued ...

CLUSTER

TEST NAME	I (7)	F (5)	G (14)	H (11)	J (5)
<b>ii) DICARBOXYLIC ACID</b>					
OXALATE	-	-	-	-	+
MALONATE	3	+	-	6	+
FORMATE	2	3	3	6	+
PIMELATE	5	+	-	1	+
SUBERATE	+	+	+	+	+
SUCCINATE	+	+	+	+	+
ADIPATE	1	+	*	-	+
AZELATE	4	-	-	4	+
SEBACATE	2	-	1	3	-
MELEATE	3	-	-	1	+
FUMERATE	+	+	+	+	+
<b>iii) HYDROXYACIDS</b>					
DL-MALATE	+	+	+	+	+
L-MALATE	-	+	3	6	-
DL-GLYCERATE	1	+	-	7	+
DL-LACTATE	+	+	+	+	+
DL- $\alpha$ -HYDROXYBUTYRATE	5	+	11	7	+
D-(-)-TARTRATE	-	-	-	3	+
L-(+)-TARTRATE	3	1	2	6	+
MESO-TARTRATE	2	-	-	5	+
GLYCOLATE	1	+	-	4	+
CITRATE	+	+	+	+	+
$\alpha$ -KETOGlutARATE	+	+	+	+	+
PYRUVATE	+	+	+	+	+
ITACONATE	-	-	-	3	+
ACONITATE	4	+	+	+	+
<b>AMINO ACIDS AND RELATED COMPOUNDS</b>					
L-HISTIDINE	4	+	+	+	+
L-PROLINE	+	+	+	+	+
L-TYROSINE	+	-	-	1	+
KYNURENATE	1	1	-	1	+
L-PHENYLALANINE	4	3	5	+	+
L-TRYPTOPHAN	1	1	-	2	+

Table 6b continued ...

CLUSTER

TEST NAME	(7)	(5)	(14)	(11)	(5)
(AMINO ACIDS AND RELATED COMPOUNDS CONTINUED)					
P-AMINOBENZOATE	-	-	-	-	+
PUTRESCINE	5	3	1	+	+
DL-KYNURENATE	1	-	-	3	+
ETHANOLAMINE	5 *	2	-	4	+
D-TRYPTORHAN	1	-	-	-	+
BENZYLAMINE	-	-	-	5 *	+
NON-NITROGENOUS AROMATIC AND OTHER CYCLIC COMPOUNDS					
D-MANDELATE	2	-	-	5	+
L-MANDELATE	-	1	-	3	+
BENZOATE	5 *	-	-	3	1
M-HYDROXYBENZOATE	1	1	-	-	3
PHthalate	1	-	-	-	+
QUINATE	5 *	-	-	3	1
P-HYDROXYBENZOATE	5 *	-	-	3	1
OTHER NITROGENOUS COMPOUNDS					
BETAIN	4	3	-	+	2
SARCOSINE	1	-	-	+	+
CREATINE	3	-	-	3	+
HIPPURATE	4	3	3	+	+
NIACINAMIDE	1	-	-	4	*
NICOTINATE	-	-	-	-	+
ALLANTOIN	2	-	-	+	*
ADENINE	1	-	-	-	*
ENZYME REACTIONS					
GELATINASE	1	-	2	3	-
AGARASE	1	-	-	-	-
ALGINASE	-	1	-	2	-
CATALASE	+	+	+	+	+
CELLULASE	3	1	-	-	+
CHITINASE	-	-	-	-	-
LAMARINASE	-	-	-	-	-
AMYLASE	+	+	+	7	+
LIPASE	-	1	-	2	-
OXIDASE	4	3	+	+	-

Table 6b continued ...

CLUSTER

TEST NAME	I (7)	F (5)	G (14)	H (11)	J (5)
<b>GROWTH TEMPERATURES</b>					
GROWTH (5 deg C)	+	+	+	+	+
GROWTH (35 deg C)	+	+	6	+	+
GROWTH (40 deg C)	-	+	3	6	-
<b>OTHER CHARACTERISTICS</b>					
DENITRIFICATION	-	+ *	-	-	-
FLUORESCENCE	-	-	-	2	-

fermentative. These organisms were able to utilize isovalerate, glycocholate, malonate, D-galactose, and isobutyrate as sole carbon sources.

Cluster E (Table 6a) consisted of 10 epibenthic OTU.

With the exception of 2 OTU, all were orange pigmented.

Although all 10 had an oxidative metabolism, 5 of these did not give a reaction in MOF medium. Only 2 OTU required sodium-ions for growth and were motile. These OTU were able to utilize adenine, itaconate, kynurename, D-mandelate, creatine, sarcosine, pimelate and L-tyrosine as sole sources of carbon. Several strains demonstrated agarase activity.

Cluster F (Table 6b) consisted of three oxidative OTU from the epibenthic zone and two type OTU. The type OTU were Pseudomonas nautica and Alicaligenes aquamarinus. All OTU showed a definite sodium-ion requirement and were motile. These OTU showed the ability to denitrify. Also, this cluster was able to utilize adipate, glycolate, n-butanol and pelargonate as sole carbon sources.

Cluster G (Table 6b) was composed of only oxidative epibenthic OTU. Eleven OTU demonstrated a definite sodium-ion requirement and all OTU were motile. A distinguishing character for this cluster was the utilization of valerate as a sole carbon source.

Cluster H (Table 6b) consisted of 10 type cultures. Eight were oxidative, 2 fermentative and one MFO negative. All demonstrated a sodium-ion requirement and were motile.

This cluster was distinguished from other clusters by the ability to utilize L-alanine, allantoin, L-ornithine, DL-citrulline, benzylamine; pelargonate, betaine and D-galacturonate as sole sources of carbon.

Cluster I (Table 5b) consisted of 1 epibenthic, 4 pelagic and one type OTU, Oceanospirillum vagum. Five OTU were fermentative, the other 2 were MFO negative. One OTU was pigmented. All OTU demonstrated motility. Two OTU required sodium-ions. These OTU were able to utilize meso-inositol, and ethanolamine as sole sources of carbon. In addition, five OTU were able to utilize benzoate, p-hydroxybenzoate and quinate as sole carbon sources. One OTU (84) utilized the above aromatic compounds via meta cleavage, the other 4 OTU were able to utilize aromatic compounds via ortho cleavage.

Cluster J (Table 5b) consisted of only strains from the second isolation. All OTU were fermentative, orange pigmented, motile and did not show any requirement for sodium-ions. The utilization of the following compounds were unique to this cluster: nicotinate, p-aminobenzoate, phthalate, D-fucose, D-tryptophan, DL-norleucine, inulin, L-rhamnose, D-tartrate, maleate, L-tryptophan, L-mandelate, oxalate, benzylamine, isopropanol, adipate, DL-citrulline, niacinamide, DL-kynurenate, valine, adenine, meso-tartrate, L-ornithine, glycolate, kynurenate, allantoin, itaconate, L-tartrate, creatine and L-lysine. OTU in this cluster grew

on a large number of substrates compared to the OTU in other clusters.

With the exception of 2 orange-pigmented OTU (OTU 52 - 53, and OTU 45 - 109) and OTU 47 (which gave an ca. 90% agreement to OTU 106), all replicates used in this study gave a minimum of 97% agreement.

In addition to the numerical analysis using Euclidean distance and Ward's clustering method, several other clustering techniques were used to determine the robustness of the clusters. A similarity measure obtained using Jaccard coefficient and average linkage clustering in an abbreviated version is shown in Fig. 6. Simple matching coefficient was used for both single linkage clustering and density search clustering. An abbreviated version of these dendograms is shown in Fig. 7 and Fig. 8 respectively. A dendrogram using euclidean distance and relocate clustering is shown in Fig. 9. An identical hierachial arrangement of the clusters was obtained using euclidean distance with both relocate and Ward's clustering methods. Similar hierachial clusters were obtained using Jaccard and simple matching coefficients. Differences between similarity and dissimilarity measures were minor. OTU which failed to cluster consistently are listed in Table 7. Most of these OTU were either type cultures or orange-pigmented OTU which had few characteristics in common with other OTU.

Figure 6. An abbreviated version of a dendrogram obtained using 135 binary tests, 115 OTU, Jaccard coefficient and average linkage clustering method. OTU which clustered with two or more OTU were labelled with the letter assigned to it in Fig 5. Numbers below cluster lettering indicates number of OTU associated in cluster. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.

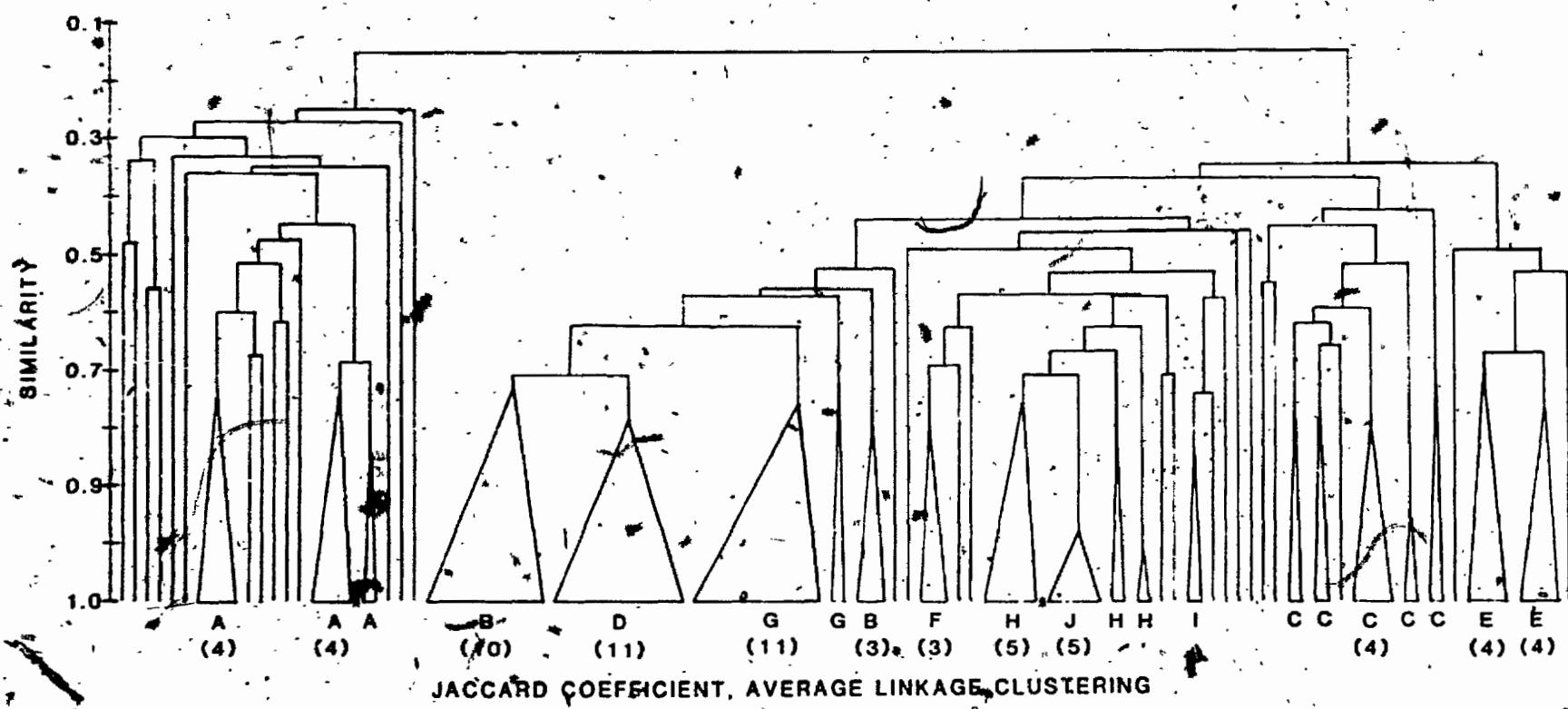


Figure 7. An abbreviated version of a dendrogram obtained using 135 binary tests, 115 OTU, simple matching coefficient and single linkage clustering method. OTU which clustered with two or more OTU were labelled with the letter assigned to it in Fig 5. Numbers below cluster lettering indicates number of OTU associated in lettering cluster. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.

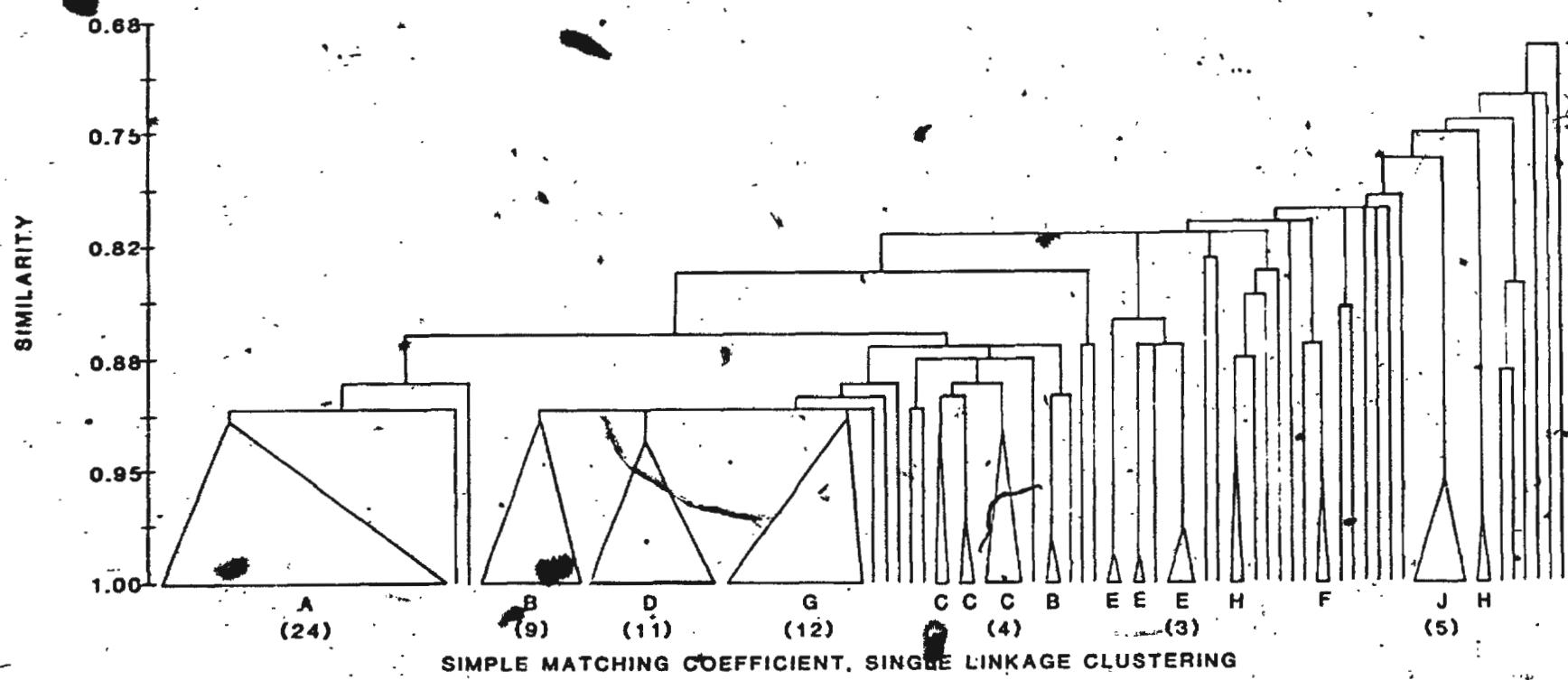
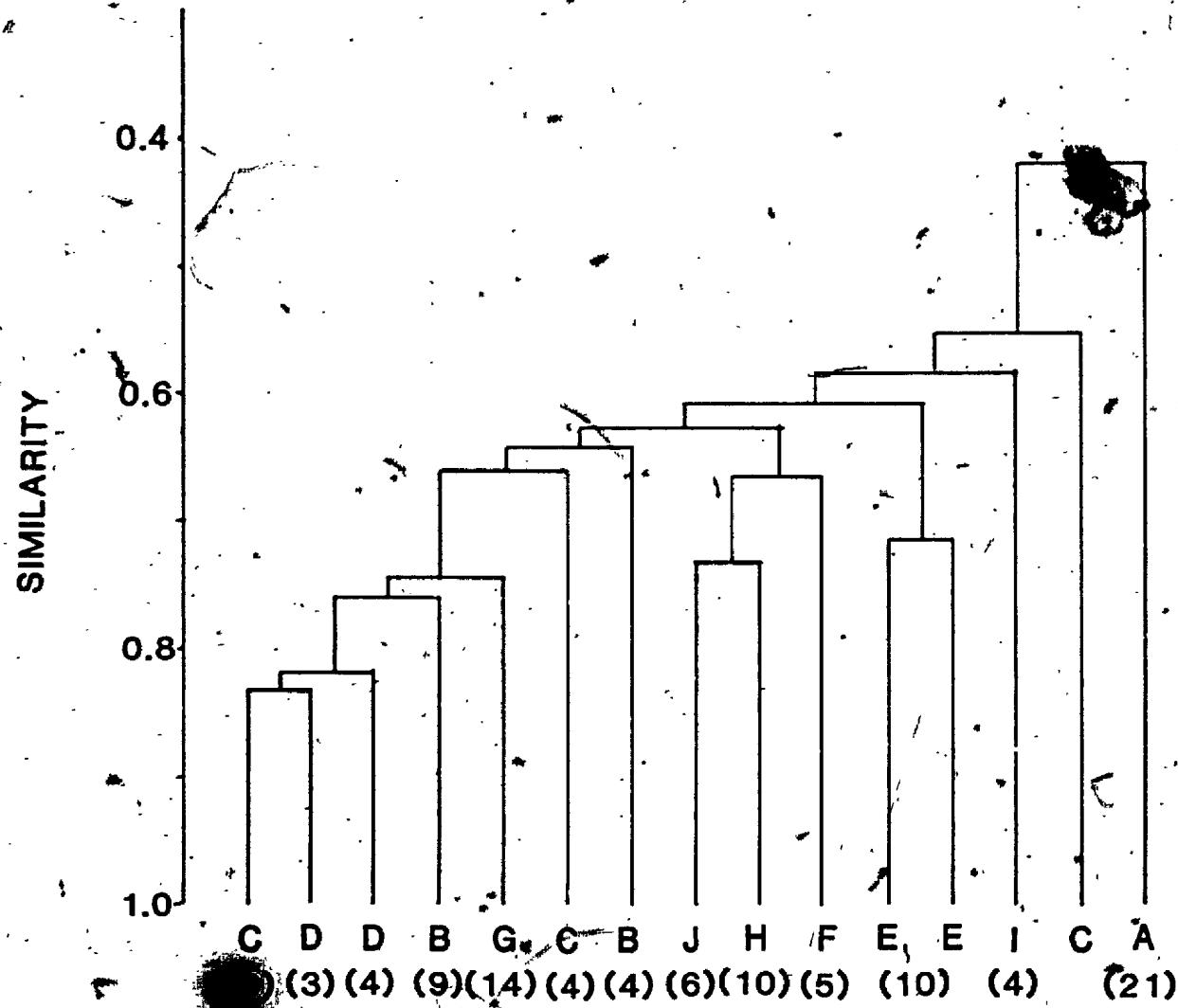


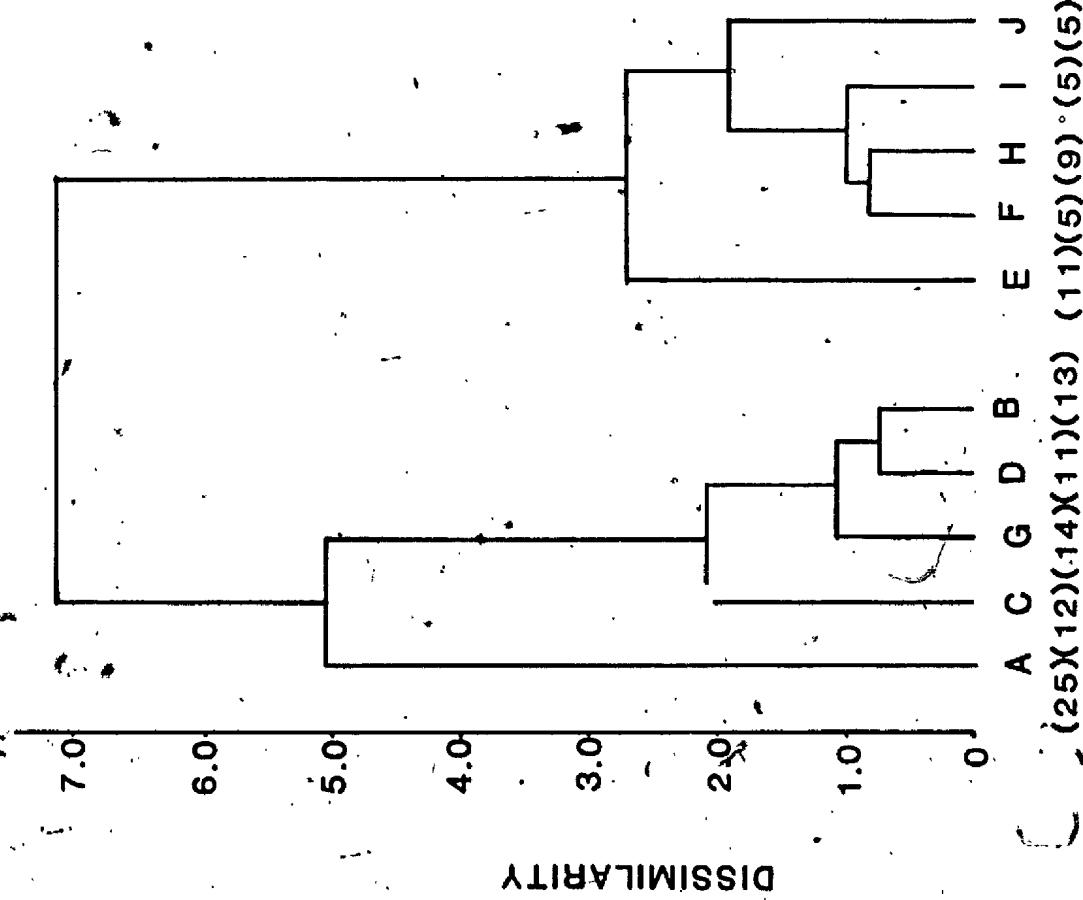
Figure 8. An abbreviated version of a dendrogram obtained using 135 binary tests, 115 OTU, simple matching coefficient and density clustering. OTU which clustered with two or more OTU were labelled with the letter assigned to it in Fig 5. Numbers below cluster lettering indicates number of OTU associated in cluster. The Y-axis gives the similarity values at which OTU and clusters of OTU merge.



SIMPLE MATCHING COEFFICIENT, DENSITY CLUSTERING

Figure 9. An abbreviated version of a dendrogram obtained using 135 binary tests, 115 OTU, Euclidean distance coefficient and relocate clustering.

Ten clusters are shown. Numbers below cluster lettering indicates number of OTU associated in cluster. The Y-axis gives the dissimilarity values at which OTU and clusters of OTU merge.



EUCLIDEAN DISTANCE, RELOCATE CLUSTERING

### 3. Identification of OTU

For the purposes of identification, 31 type cultures and 10 reference OTU were added to the previously constructed data matrix. These were listed in Table 2 and 3. Then, fermentative OTU were separated from non-fermentative OTU. A list of the 114 biochemical tests with corresponding test number is found in Table 1, Appendix B. The data matrix for fermentative and non-fermentative OTU and characters is seen in Tables 2 and Table 3 respectively in Appendix B.

Simple matching with single linkage clustering was used to determine the relationship between type and reference strains. In total, 114 characteristics and 85 fermentative OTU were investigated. One hundred and fourteen characteristics were used with 72 non-fermentative OTU.

The type and reference cultures were used to help identify the OTU. In addition it was necessary to use published determinative schemes to complete the identification. The schemes used were from the 9th edition of Bergey's Manual of Systematic Bacteriology (Krieg, 1984). These are given in Table 8.

#### i) Fermentative OTU

The dendrogram obtained by simple matching coefficient with single linkage clustering is shown in Fig. 10. At a

Table 7. OTU which did not cluster consistently.  
Shown is the region of isolation and  
corresponding OTU designation of strain.

OTU	Source	isolation~
1	pelagic	1st
3	pelagic	1st
15	pelagic	1st
27	pelagic	1st
30	benthic	1st
31	benthic	1st
34	benthic	1st
37	benthic	1st
44	benthic	1st
53	benthic	1st
58	benthic	1st
59	benthic	1st
66	type	
69	type	
70	type	
71	type	
74	type	
75	type	
76	type	
78	type	
81	pelagic	2nd
97	pelagic	
108	benthic	1st
111	type	

: 1st isolation (Nov., 1982) or 2nd isolation (June, 1983)

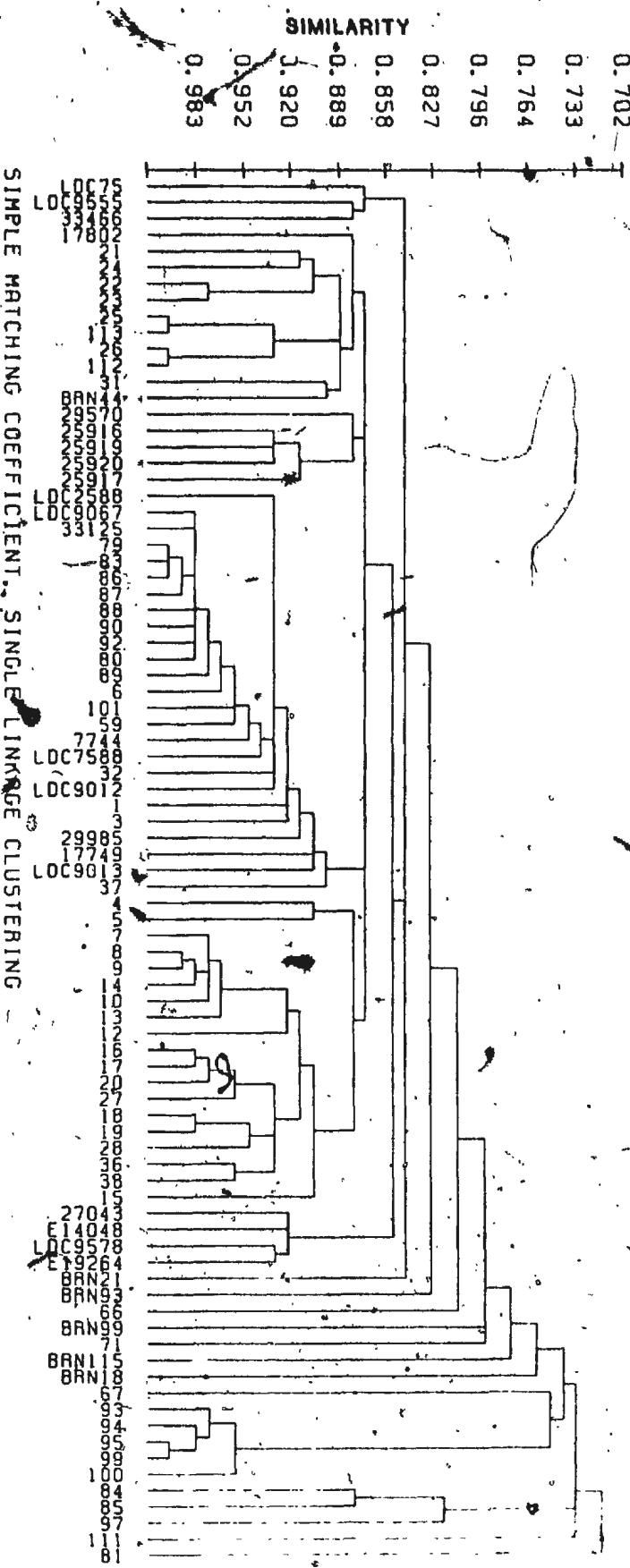
dissimilarity value of ca. 0.87, 7 clusters were obtained. The first cluster consisted of the OTU 21, 22, 23, 24, 25, 26, 31, 112 and 113 with a type culture Vibrio parahemolyticus (ATCC 17802) and BRN44, a reference Vibrio isolated by Hollohan (1982). These OTU were all constituents of cluster C (Table 5) which required sodium-ions, were motile with polar flagella, oxidase positive, and able to utilize D-glucose, maltose and D-mannitol as sole sources of carbon. These OTU were from both the pelagic and epibenthic regions from the first isolation. Reference strain BRN44 from Alaria esculenta, clustered with OTU 31 at a similarity value of 0.90. V. parahemolyticus (ATCC 17802) clustered at a 0.88% similarity with this cluster. All strains in this cluster were tentatively identified as Vibrio.

A second cluster consisted of OTU from cluster A (Table 5) and Vibrio type cultures. At a similarity value of 0.98, Vibrio alonsis (LDC 9067) and Vibrio proteolyticus (ATCC 33125) clustered with OTU 79, 80, 86, 87, 88, 90 and 92. These OTU were originally part of cluster A and consisted of pelagic strains from the June, 1983 isolation. Not all of the pelagic OTU had a sodium-ion requirement but all were motile. None was able to utilize D-fructose. All were oxidase negative. Because these lacked a sodium-ion requirement they were not identified as Vibrio sp. These OTU were identified as Aeromonas. OTU 1, 3, 6, 32, 37, 59

Table 8: Sources of the diagnostic schemes used to identify pelagic and epibenthic OTU from the Northwest Atlantic Ocean near Newfoundland.

Genus	Reference
<u>Aeromonas</u>	Popoff (1984); Baumann and Schubert (1984).
<u>Alteromonas</u>	Baumann, Gauthier and Baumann (1984).
<u>Flavobacterium</u>	Holmes, Owen and McMeekin (1984).
<u>Pseudomonas</u>	Palleroni (1984).
<u>Vibrio</u>	Baumann, Furness and Lee (1984); Baumann and Schubert (1984).

Figure 10. Dendrogram obtained using 114 binary tests,  
85 fermentative OTU, simple matching  
coefficient and single linkage clustering method.  
The Y-axis gives the similarity values at which  
OTU and clusters of OTU merge.



and 89 were pelagic and epibenthic strains from November, 1982 and June, 1983 isolations. These OTU clustered with the latter OTU as well as the type strains Vibrio fischeri (ATCC 7744), Vibrio damsela (LDC 7588), Vibrio logei (ATCC 29985), and Vibrio costicola (LDC 9013) at a 0.90 similarity value. All except OTU 32 and 89, which did not require sodium-ions, were identified as Vibrio sp. OTU 32 was oxidase positive, motile, able to utilize D-glucose, D-fructose and D-mannitol and therefore had many of the properties of Aeromonas. OTU 89 could not be identified.

The next cluster consisted predominately of OTU from cluster D (Table 5), but several OTU from clusters B (Table 5) and C (Table 5) were also present. Type or reference strains did not cluster with this group. All OTU in this cluster required sodium-ions, were motile, oxidase positive, utilized D-glucose and D-fructose. Only OTU 13 was unable to utilize D-mannitol.

OTU 7, 10, 12, 13, 16, 17, 19, 20 and 27 were able to utilize glycerol. With the exception of OTU 7, 8, 9, 10 and 14, all were able to utilize maltose as a sole source of carbon. Lipase activity was apparent in OTU 4, 5, 12, 17, 20, 24 and 28. These OTU did not accumulate PBB, were polarly flagellated and required sodium-ions. They were tentatively identified as Vibrio.

Cluster J (Table 5) was the tightest cluster at a similarity value of 0.952. This had pelagic OTU from the

June, 1983 isolation. These OTU showed no sodium-ion requirement, were oxidase negative, orange pigmented, able to utilize a wide variety of carbon compounds, and one was motile with two polar flagella. Tentative identification of these OTU was difficult because they were oxidase negative. This suggested that these OTU were neither Vibrio nor Aeromonas. Nor could they be classify as Flavobacterium because they were fermentative, motile, displayed swarming activity and were oxidase negative. These OTU could not be assigned to the genus Cytophaga because this genus was oxidative and oxidase positive. This cluster remained unidentified.

OTU 84 and 85 were found in cluster I. Both did not require sodium-ions. However both OTU utilized D-mannitol, D-glucose, D-fructose and were catalase positive and motile. OTU 85 was able to utilize glycerol and was oxidase positive. OTU 85 was tentatively identified as Aeromonas. OTU 84 could not be identified.

ii) Non-fermentative OTU; oxidative metabolism.

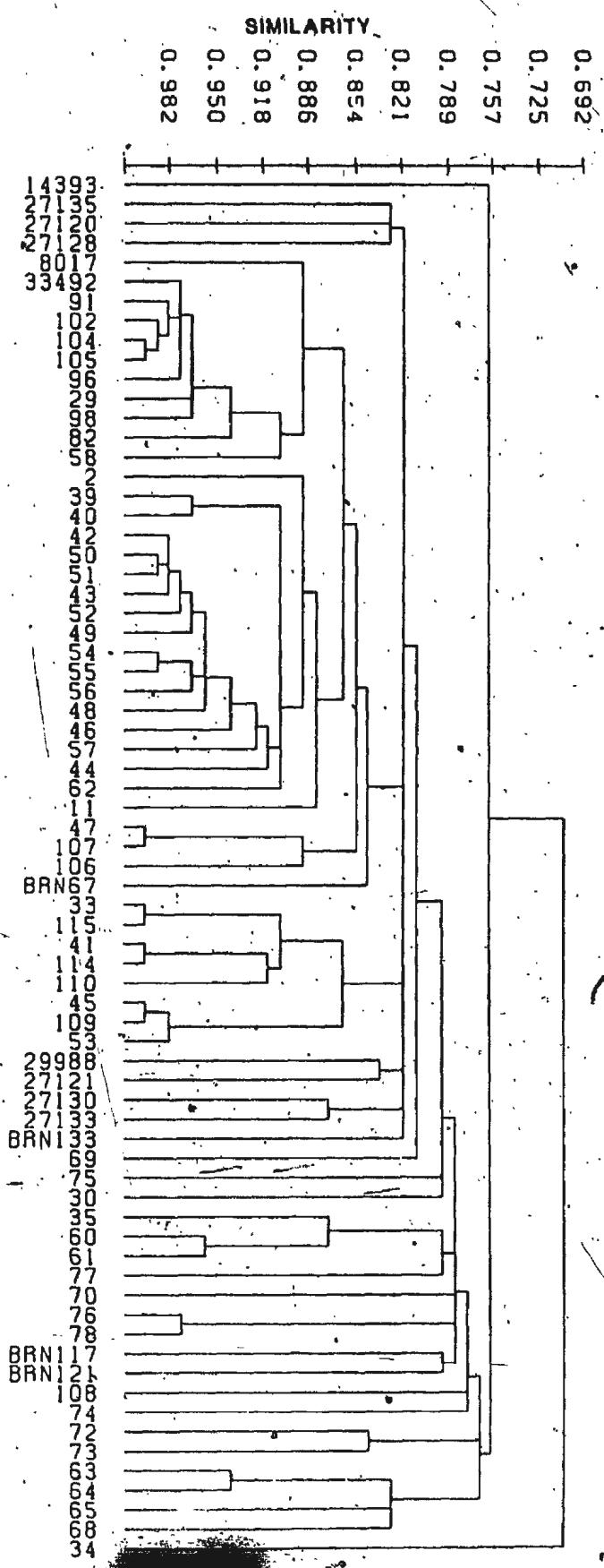
The dendrogram obtained by simple matching coefficient with single linkage clustering is shown in Fig. 11. At ca. 0.85 similarity value, several clusters were formed. The first cluster consisted of OTU 2, 11, 29, 39, 40, 42, 43, 44, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 62, 82, 91, 96, 98, 102, 104, 105, 106 and 107 from clusters A, B

and G.

OTU from cluster A (Table 5), subclustered at a similarity value of ca. 0.90. OTU 91, 96, 102, 104 and 105 were June strains which had a 0.98 similarity to type culture Alteromonas luteoviolacea (ATCC 33492). OTU 29, 58, 82 and 91 had a 0.89 similarity to type culture Alteromonas putrefaciens (ATCC 8017). With the exception of OTU 29, all required sodium-ions and were motile. All utilized D-glucose except for the OTU 29, 82, 91 and 96. OTU 82 utilized D-fructose as a sole source of carbon. OTU 29, 102 and 104 utilized DL-malate. This cluster was identified as belonging to the genus Alteromonas.

Another subcluster was formed by OTU 2, 11, 39, 40, 42, 50, 51, 43, 44, 46, 48, 49, 54, 55, 56, 57 and 62, at a similarity value of 0.88. OTU in this subculture were mostly from cluster G (Table 5) and consisted of strains from the pelagic and epibenthic regions collected in November, 1982. These OTU were unable to utilize D-xylose, L-arabinose, D-fucose, L-rhamnose, melibiose, lactose, D-gluconate, galacturonate, oxalate, maleate, D-( $\text{--}$ )tartrate, meso-tartrate, isopropanol, DL-norleucine, L-citrulline, DL- $\alpha$ -aminobutyrate, D-aminobenzoate, benzylamine, nicotinate, nicotinamide, D-sorbitol, m-hydroxybenzoate or produce the extracellular enzyme alginase. All OTU were able to utilize acetate, DL-lactate and pyruvate as sole carbon sources. With the exception of

Figure 11. Dendrogram obtained using 114 binary tests,  
71 non-fermentative OTU, simple matching  
coefficient and single linkage clustering method.  
The Y-axis gives the similarity values at which  
OTU and clusters of OTU merge.



SIMPLE MATCHING COEFFICIENT, SINGLE LINKAGE CLUSTERING

OTU 44, all OTU were able to utilize DL-malate. Only OTU 52, 57 and 62 were unable to utilize gluconate. All OTU were polarly flagellated, motile and with the exception of OTU 52, 57 and 62, required sodium-ions. These OTU were tentatively assigned to the genus Pseudomonas.

The final subcluster consisted of OTU 47, 106 and 107 which were oxidative stains from cluster B (Table 5). All were benthic strains and required sodium-ions for growth. These OTU were able to utilize L-malate, D-sorbitol, m-hydroxybenzoate, glucose, cellobiose, and propionate. All were oxidase positive and utilized aromatic compounds via ortho cleavage. These OTU could not utilize acetate, but were able to utilize D-glucose and D-fructose. This group of OTU was identified as Alteromonas.

At a similarity level of ca. 0.86, reference culture BRN67 (Alteromonas) fused with these OTU. Reference culture BRN67 was isolated from the seaweed Alaria esculenta.

OTU 33, 41, 45, 57, 109, 110, 114 and 115 were all epibenthic strains originally in cluster E (Table 5). All OTU in this cluster were able to hydrolyse starch. With the exception of OTU 41 and 114, all OTU in this cluster were orange pigmented and non-motile. OTU 45, 47, 109 and 110 were tentatively identified as Flavobacterium. Because OTU 33 and 115 demonstrated agarase activity they could not be assigned to the genus.

OTU 35, 60 and 61 were epibenthic strains and represent

OTU from cluster F (Table 5). None of these OTU were able to utilize D-xylose, D-fucose, L-rhamnose, D-mannose, melibiose, lactose, inulin, salicin, n-acetylglucosamine, oxalate, maleate, D-( $-$ )-tartrate, meso-inositol, adonitol, DL-norleucine, L-citrulline, DL- $\alpha$  aminobutyrate, p-aminobenzoate, benzylamine, nicotinate, and nicotinamide. None of these OTU produced the extracellular enzymes alginase or chitinase but were able to demonstrate the ability to denitrify. All the OTU were able to utilize acetate, DL-lactate, pyruvate, D-glucose and gluconate as sole carbon sources. Since all OTU required sodium-ions and were motile with polar flagellation, this group of OTU were tentatively identified as Pseudomonas.

In summary, identifications at the generic level were made and are presented in Table 9. Fermentative OTU were Vibrio and Aeromonas. In general, Vibrio species were apparent in not only epibenthic regions of the water column, but in the pelagic regions during both the November and June sampling period. Aeromonas was tentatively identified in the pelagic zone from the June, 1983 sampling period and an epibenthic strain taken during the November, 1982 sampling period. Orange pigmented Flavobacterium species were also recovered from the epibenthic region. However, pigmented bacteria were not limited to the epibenthic regions. Several pigmented strains were apparent in the pelagic zone during the June isolation. A small group of orange

Table 9. Summary of tentatively identified OTU.

Shown is the Operational Taxonomic Unit (OTU) number, the type strain(s) used to assist identification (Type), region and date of isolation of OTU, and the tentative identification of OTU. November, 1982 and June, 1983 isolates are coded as 'Nov.' and 'June' respectively.

OTU No.	Type	Date isolated	Region of isolation	Tentative identification
21,22, 23,	BRN44	Nov.	pelagic	
24,25, 26,	Vibrio			Vibrio
113, 112	<i>parahemolyticus</i>		epibenthic	
3, 6,	<i>V. fischeri</i>		pelagic	Vibrio
32,37	<i>V. damsella</i>			
	<i>V. logei</i>	Nov.	epibenthic	
	<i>V. costicola</i>			
4,5,7,8,				
9, 10,12,			pelagic	Vibrio
13,14,15,		Nov.		
16,17,18,			epibenthic	
19,20,28,				
36,38				
32	-	Nov.	epibenthic	<u>Aeromonas</u>
79,80,85,86,	-	June	pelagic	<u>Aeromonas</u>
87,88,90,92				
35,60,61	-	Nov.	epibenthic	<u>Pseudomonas</u>
2,11,39,				
40,42,43,	-		pelagic	
44,46,48,				
49,50,51,	-	Nov.	epibenthic	<u>Pseudomonas</u>
54,55,56				
45,47,109,	-	Nov.	epibenthic	<u>Flavobacterium</u>
110				
47,106,107	-	Nov.	epibenthic	<u>Alteromonas</u>
29,58,82,	<u>Alteromonas</u>			
91,96,102,	<u>luteoviolacea</u>	June	pelagic	<u>Alteromonas</u>
104,105	<u>A. putrefaciens</u>			
93,94,95,	-	June	pelagic	unidentified
99,100				

pigmented bacteria which showed fermentative properties were also recovered during this isolation period. These strains could not be identified although they utilized a very large number of organic compounds as sole sources of carbon and energy. Several strains of Pseudomonas were identified. These species occurred in epibenthic zones. Alteromonas was recovered from epibenthic samples.

### Discussion

Marine bacteria which readily become attached, metabolize and form colonies on high nutrient sources are known as heterotrophs or eutrophs (Fukami *et al.*, 1983). In this study, bacteria were isolated from sea-water by using nutrient agar containing 2 g organic carbon per litre. These bacteria would be heterotrophic rather than oligotrophic (Akagi *et al.*, 1977).

Samples were taken from a cold ocean environment, and were assumed to contain either psychophilic or psychrotrophic bacteria. Gow and Mills (1984) have shown that these groups predominate in the water of the Northwest Atlantic Ocean near Newfoundland.

The methods of isolation and media used to isolate strains, have differed in previous studies. For example, Pfister and Bulkholder (1965) isolated bacteria using a medium consisting of trypticase, soytone, yeast extract (YE) and vitamin B(12) in aged seawater. Other studies such as those of Quigley and Colwell (1968), Singleton and Sneath (1973), Kaneko *et al.* (1979), Hauxhurst *et al.* (1980) and this study have used the media consisting of peptone, YE, minerals and agar while Austin *et al.* (1979) used a variety of media to isolate strains. Some investigators have used pour-plate method to isolate strains (Pfister and Bulkholder, 1965), while other researchers have chosen to

first dilute water samples in sea-water nutrient broth, and then plate samples on nutrient agar (Singleton and Sneath, 1973). Provided that these limitations are recognized a comparison of genera from different regions can be made.

In this study, fermentative bacteria were tentatively identified as Vibrio although some species of the genus Aeromonas were present. Nonfermentative strains were identified as Alteromonas, Flavobacterium and Pseudomonas. Species of the genera Photobacterium, Chromobacterium, Arthrobacter, Acinetobacter-Morexalla or Cytophaga were not found in this study.

Approximately 23% and 9% of the genera isolated in this study were identified as Vibrio and Aeromonas respectively. The Pfister and Burkholder (1965) study of bacteria from the Antarctic and tropical oceans did not show either of these genera. Quigley and Colwell (1968) found that ca. 7% of their strains from the Pacific Ocean were Aeromonas. An additional 7% of their strains were either Vibrio or Aeromonas. Singleton and Sneath (1973) found ca. 48% of strains from coastal New Zealand were Vibrio. Approximately 16% of the strains from the Austin et al. (1974) study were Vibrio. Hauxhurst et al. (1980) found that 48 and 46% of the strains from the eastern and western Gulfs of Alaska were respectively, Vibrio. Although no species of the genus Aeromonas were found in the western Gulf of Alaska, an additional 22% of the strains from the eastern Gulf of

Alaska could have been either Vibrio or Aeromonas.

Therefore, the proportions of Vibrio from the region examined in this study most closely resembled those reported from the ~~western~~ Gulf of Alaska (Hauxhurst *et al.*, 1980) and from the coastal waters of New Zealand as described by Singleton and Sneath (1973).

Approximately 22% and 12% of the strains in this study were identified as Pseudomonas and Alteromonas respectively. Only 2% of strains were identified as Flavobacterium.

Because the name Alteromonas was not proposed until recently (Baumann *et al.*, 1972), many studies have included both Pseudomonas and Alteromonas as the single genus Pseudomonas.

Examples are the studies of Pfister and Burkholder (1965), Quigley and Colwell (1968), and Singleton and Sneath (1973).

Approximately 32% and 2% of the strains from the Pfister and Burkholder (1965) study were identified as Pseudomonas and Flavobacterium respectively. Quigley and Colwell (1968) and Singleton and Sneath (1973) found 8% and 11% of their strains respectively were Pseudomonas. Although 40% of the strains could not be assigned to any genus by Austin *et al.* (1979), ca. 7% of the strains were assigned to the genus Pseudomonas. Kaneko *et al.* (1979) found 44% of their strains were Flavobacterium while only 3% of their strains were either Pseudomonas, Alteromonas or Alcaligenes.

Although Hauxhurst *et al.* (1980) did not find any Alteromonas sp., they did find that Flavobacterium and

Pseudomonas were 1% and 6% of the strains respectively, from the eastern Gulf of Alaska. No species of the genus Pseudomonas were found in the western Gulf of Alaska but, 19% of the strains were either Flavobacterium or Cytophaga. Therefore, in this study, the proportion of Pseudomonas, Alteromonas and Flavobacterium resembled those reported by Pfister and Burkholder (1965) if it is taken into account that Pfister and Burkholder (1965) would not have differentiated between Pseudomonas and Alteromonas. The proportion of Alteromonas was slightly higher than reported by other investigators. This genus has previously been found in Newfoundland waters (Hollohan, 1982).

Vibrio identified in this study could be divided into three groups. One group of strains utilized several carbon sources and was able to produce many extracellular enzymes. Unlike two other groups of vibrio, these strains were not found in the epibenthic regions of the water column. Another group occurred in both the pelagic and epibenthic regions and did not produce any extracellular enzymes or utilize many of the carbon sources tested. The third group from the pelagic and epibenthic regions, was able to produce one or more extracellular enzymes as well as utilize several carboxylic acids.

Pseudomonas sp. could be divided into two groups. One group of pseudomonads utilized few carbohydrates, alcohols and amino acids. The other group was able to denitrify and

utilize several alcohols and carboxylic acids as sole carbon sources.

One group of Alteromonas demonstrated ortho cleavage of aromatic compounds. The other group of Alteromonas did not demonstrate the ability to cleave aromatic compounds or utilize many of the carbon sources tested.

Flavobacterium, which are pigmented, and another group of unnamed orange-pigmented strains, did not share similar physiological or nutritional characteristics.

Flavobacterium was oxidative, non-motile and nutritionally fastidious. Conversely, the orange-pigmented unknown strains were fermentative, motile and able to utilize most of the carbon sources tested.

Problems were encountered when subculturing of bacterial strains, but this is not uncommon. It has been reported in other studies (Boeye et al., 1975; Oliver et al., 1982). In this study, less than 30% of strains initially isolated were viable six weeks after initial isolation. Poor viability was observed in pigmented and non-pigmented strains. Some pigmented strains showed little or nonexistent growth on single carbon substrates. Kaneko et al. (1979) and Hauxhurst et al. (1980) noted the requirement of vitamins and growth factors for some strains in their studies. Vitamins or growth factors may be needed for optimal growth of some bacteria from this region as well.

In general, pelagic strains differed from epibenthic strains. Pelagic strains, unlike epibenthic strains, demonstrated the ability to produce at least one or more extracellular enzymes. Most of the pelagic strains were fermentative (80%), and consisted of the genera Vibrio and Aeromonas. Although Vibrio and Aeromonas were found in the epibenthic regions, most (83%) epibenthic strains had an oxidative metabolism and consisted of the genera Flavobacterium, Alteromonas and Pseudomonas. Although Flavobacterium was found only in the epibenthic region, Alteromonas and Pseudomonas were found in both the pelagic and epibenthic regions of the water column.

Two reference cultures from Hollohan (1982) showed similarities to strains isolated in this study. Reference culture BRN44 identified as a Vibrio was isolated from the seaweed Alaria esculenta. This culture merged with vibrios from the pelagic region described in this study. Reference culture BRN67, which was an Alteromonas, was also from Alaria esculenta. This culture strain merged with strains derived from both the pelagic and epibenthic regions of the water column. These results suggests that Alteromonas is widely distributed in the marine environment in this region.

The abundance of oxidative and lack of fermentative bacteria in the epibenthic regions of the water column is of particular interest. It might be suspected that below the mud-water interface, anoxic conditions would provide an

optimal environment for fermentative bacteria. But, anoxic conditions can begin at depths of ca. 40 cm and greater (Kepkay and Novitsky, 1980). The epibenthic zone (in theory), consists of a thin layer of particulate matter, sometimes referred to as the mud-water slurry (Quigley and Colwell, 1967) or flocculent material (Novitsky, 1983a). Metabolic activity in these regions is very high when compared to the rest of the water column (Chocair and Albright, 1980). Many active cells are able to react immediately to incoming nutrient sources such as detritial matter filtering through the overlying waters (Novitsky, 1983a; 1983b).

For this study it was shown that the epibenthic region predominantly consisted of oxidative bacteria such as the genera Flavobacterium, Pseudomonas and Alteromonas. With the exception of the genus Flavobacterium, these bacteria also occurred in the overlying waters but not in the same relative proportion. Bacteria from both the epibenthic and pelagic regions were able to utilize a number of organic compounds as sole sources of carbon and energy. This should benefit their chances of survival in an aquatic environment. Most of the bacteria that produced biodegradative exoenzymes were found in the pelagic region and not in the epibenthic region. It might be expected that they would also be found in the sediment which should be a rich source of substrate polymers. This could be the subject of a further

investigation to determine if the results of this study are typical and, if so, to determine why the exoenzyme producing bacteria are found predominately in the pelagic zone.

Conclusions

- 1) Pelagic strains from the marine environment were mainly fermentative.
- 2) Epibenthic strains from the marine environment were mainly oxidative and clustered separately from pelagic strains.
- 3) Fermentative stains were identified as Vibrio although some species of the genus Aeromonas were present.
- 4) Nonfermentative strains were identified as Alteromonas, Flavobacterium and Pseudomonas.
- 5) The relative proportions of the genera were not identical to those reported in other studies but most closely resembled populations of bacteria in the western Gulf of Alaska, New Zealand, and Antarctica.

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**APPENDIX A**

**Table 1: Source and date of isolation, and culture collection number (CC No.)~, isolation depth and operational taxonomic unit (OTU) designation' for each strain. OTU which were replicated are shown with the letter A or B directly following the CC No.**

OTU	CC No.	(a)		OTU	CC No.	Source	Date
		Source	Date				
1	2001	20	Nov.'82	31	D310	BENTHIC	Nov.'82
2	2002	20	Nov.'82	32	D311	BENTHIC	Nov.'82
3	2003	20	Nov.'82	33	D312A	BENTHIC	Nov.'82
4	2011	20	Nov.'82	34	D32	BENTHIC	Nov.'82
5	2019	20	Nov.'82	35	E303	BENTHIC	Nov.'82
6	2020	20	Nov.'82	36	E311	BENTHIC	Nov.'82
7	2032	20	Nov.'82	37	I310	BENTHIC	Nov.'82
8	2033	20	Nov.'82	38	I318	BENTHIC	Nov.'82
9	2034	20	Nov.'82	39	S31	BENTHIC	Nov.'82
10	2037	20	Nov.'82	40	S312	BENTHIC	Nov.'82
11	6001	60	Nov.'82	41	S317A	BENTHIC	Nov.'82
12	6003	60	Nov.'82	42	S319	BENTHIC	Nov.'82
13	6004	60	Nov.'82	43	S320	BENTHIC	Nov.'82
14	6005	60	Nov.'82	44	M31	BENTHIC	Nov.'82
15	6006	60	Nov.'82	45	M315A	BENTHIC	Nov.'82
16	6007	60	Nov.'82	46	M331	BENTHIC	Nov.'82
17	6008	60	Nov.'82	47	M334A	BENTHIC	Nov.'82
18	6010	60	Nov.'82	48	Z42	BENTHIC	Nov.'82
19	6011	60	Nov.'82	49	Z44	BENTHIC	Nov.'82
20	6012	60	Nov.'82	50	Z418	BENTHIC	Nov.'82
21	6013	60	Nov.'82	51	Z442	BENTHIC	Nov.'82
22	6014	60	Nov.'82	52	Z440A	BENTHIC	Nov.'82
23	6015	60	Nov.'82	53	Z440B	BENTHIC	Nov.'82
24	6018	60	Nov.'82	54	Z457	BENTHIC	Nov.'82
25	6019A	60	Nov.'82	55	Z458	BENTHIC	Nov.'82
26	6020A	60	Nov.'82	56	Z459	BENTHIC	Nov.'82
27	6021	60	Nov.'82	57	N1	BENTHIC	Nov.'82
28	6022	60	Nov.'82	58	N15	BENTHIC	Nov.'82
29	D33	BENTHIC	Nov.'82	59	N43	BENTHIC	Nov.'82
30	D39	BENTHIC	Nov.'82	60	N41	BENTHIC	Nov.'82

~ : Culture collection number assigned to the strain.

: Number used in this study.

(a): Samples were taken at either 20m, 60m depth or from the surface of the benthic zone (epibenthic).

(Table 1 continued)

OTU	CC No.	Source	Date	OTU	CC No.	Source	Date
61	N42	BENTHIC	Nov.'82	91	14	60	June'83
62	N75	BENTHIC	Nov.'82	92	15	60	June'83
63	27214	TYPE		93	16	60	June'83
64	27374	TYPE		94	17	60	June'83
65	27123	TYPE		95	18	60	June'83
66	25915	TYPE		96	19	60	June'83
67	25914	TYPE		97	20	60	June'83
68	27126	TYPE		98	21	60	June'83
69	27125	TYPE		99	22	60	June'83
70	29660	TYPE		100	23	60	June'83
71	11040	TYPE		101	24	60	June'83
72	29659	TYPE		102	25	60	June'83
73	27118	TYPE		103	26	60	June'83
74	27119	TYPE		104	30	60	June'83
75	27132	TYPE		105	31	60	June'83
76	E13043A	TYPE		106	M334B	BENTHIC	Nov.'82
77	14400	TYPE		107	M333	BENTHIC	Nov.'82
78	E13043B	TYPE		108	M313A	BENTHIC	Nov.'82
79	1	60	June'83	109	M315B	BENTHIC	Nov.'82
80	2	60	June'83	110	M313B	BENTHIC	Nov.'82
81	3	60	June'83	111	27562	TYPE	
82	5	60	June'83	112	6020B	60	Nov.'82
83	6	60	June'83	113	6019B	60	Nov.'82
84	7	60	June'83	114	S317B	BENTHIC	Nov.'82
85	8	60	June'83	115	D312B	BENTHIC	Nov.'82
86	9	60	June'83				
87	10	60	June'83				
88	11	60	June'83				
89	12	60	June'83				
90	13	60	June'83				

Table 2. List of 135 binary tests with corresponding test number, used for numerical analyses of 115 OTU.

Test number	Test
1	$\alpha$ -AMINOVALERATE
2	ACETATE
3	ACONITATE
4	ADENINE
5	ADONITOL
6	AGARASE
7	ADIPATE
8	$\delta$ -AMINOVALERATE
9	$\alpha$ -KETOGLUTARATE
10	L-RHAMNOSE
11	ALGINASE
12	ALLANTOIN
13	ARGININE DEHYDROLASE
14	L-ARGININE
15	AZELATE
16	$\beta$ -ALANINE
17	BENZOATE
18	BENZYLAMINE
19	BETAINE
20	BUTYRATE
21	CATALASE
22	CELLULASE

Table 2 continued ...

Test number	Test
-------------	------

- |    |                 |
|----|-----------------|
| 23 | CHITINASE       |
| 24 | CITRATE         |
| 25 | CREATINE        |
| 26 | D-CELLOBIOSE    |
| 27 | D-FUCOSE        |
| 28 | D-GALACTOSE     |
| 29 | D-GLUCOSE       |
| 30 | D-MELIBIOSE     |
| 31 | D-TREHALOSE     |
| 32 | D-XYLOSE        |
| 33 | D-(-)-TARTRATE  |
| 34 | D-ALANINE       |
| 35 | D-FRUCTOSE      |
| 36 | D-GALACTURONATE |
| 37 | GLUCONATE       |
| 38 | D-GLUCURONATE   |
| 39 | D-MANDELATE     |
| 40 | D-MANNITOL      |
| 41 | D-MANNOSE       |
| 42 | D-RIBOSE        |
| 43 | D-SACCHARATE    |
| 44 | D-SORBITOL      |
| 45 | D-TRYPTOPHAN    |

Table 2 continued ...

Test number	Test
46	DENITRIFICATION
47	DL- $\alpha$ -AMINO-BUTYRATE
48	DL-ASPARTATE
49	DL- $\beta$ -HYDROXYBUTYRATE
50	DL-GLYCERATE
51	DL-KYNURENATE
52	DL-LACTATE
53	DL-MALATE
54	DL-NORLEUCINE
55	ERYTHRITOL
56	ETHANOLAMINE
57	ETHANOL
58	FERMENTATION
59	FLUORESCENCE
60	FORMATE
61	FUMARATE
62	$\gamma$ -AMINOBUTYRATE
63	GELATINASE
64	GLYCEROL
65	GLYCINE
66	GLYCOLATE

Table 2 continued ...

Test number	Test
67	GLYCOCHOLATE
68	GROWTH(5 deg C)
69	GROWTH(35 deg C)
70	GROWTH(40 deg C)
71	HEPTANOATE
72	HIPPURATE
73	INOSITOL
74	INULIN
75	ISOBUTYRATE
76	ISOPROPANOL
77	ISOVALERATE
78	ITACONATE
79	KYNURENATE
80	L-ARABINOSE
81	L-MANDELATE
82	L-(+)-TARTRATE
83	L-ALANINE
84	DL-CITRULLINE
85	L-GLUTAMATE
86	L-HISTIDINE

Table 2 continued ...

Test number	Test
87	L-ISOLEUCINE
88.	L-LEUCINE
89	L-LYSINE
90	L-MALATE
91	L-PHENYLALANINE
92	L-ORNITHINE
93	L-PROLINE
94	L-SERINE
95	L-THEORINE
96	L-TRYPTOPHAN
97	L-TYROSINE
98	LACTOSE
99	LAMARINASE
100	MALONATE
101	M-HYDROBENZOATE
102	MALTOSE
103	MELEATE
104	MESO-TARTRATE
105	MUCATE
106	N-ACETYL-D-GLUCONATE
107	N-BUTANOL

Table 2 continued ...

Test number	Test
108	NFACINAMIDE
109	NICOTINATE
110	PELARGONATE
111	OXALATE
112	OXIDATION
113	P-AMINOBENZOATE
114	P-HYDROBENZOATE
115	PHTHALATE
116	PIMELATE
117	PROPANOL
118	PROPRIONATE
119	PUTRESCINE
120	PYRUVATE
121	QUINATE
122	SALICIN
123	SARCOSINE
124	SEBACATE
125	AMYLASE
126	SUBERATE
127	SUCCINATE

Table 2 continued ...

Test number	Test
128	SUCROSE
129	LIPASE
130	VALERATE
131	VALINE
132	VOGES-PROSKAUSER
133	OXIDASE
134	MOTILITY
135	SODIUM REQUIREMENT

Table 3: Data matrix for the numerical classification.

**Table 3 (continued...)**

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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-

**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
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...)

Table 3 (continued...)

**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...)

OTU #	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...)**

Table 3 (continued...)

**Table 3 (continued...)**

**Table 3 (continued...)**

Table 3 (continued...)

Table 3 (continued...)

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	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-

**Table 3 . (continued...)**

Table 3 (continued...)

**Table 3 (continued...)**

Table 3 (continued...)

OTU #	TEST #											
	113	114	115	116	117	118	119	120	121	122	123	124
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...)

Table 3 (continued...)

Table 3 (continued...)

OTU	TEST #										
	125	126	127	128	129	130	131	132	133	134	135
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 #										
	125	126	127	128	129	130	131	132	133	134	135
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 #										
#	125	126	127	128	129	130	131	132	133	134	135
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	+	-	-	+	-	-	-	-	-	-	-

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**APPENDIX B**

Table 1. List of 114 binary tests with corresponding test number used for analyses of both fermentative and non-fermentative OTU.

Test number	Test
1	ACETATE
2	ADENINE
3	ADONITOL
4	ADIPATE
5	$\alpha$ -AMINOVALERATE
6	$\alpha$ -KETOGLUTARATE
7	L-RHAMNOSE
8	ALGINASE
9	ALLANTOIN
10	L-ARGININE
11	AZELATE
12	$\beta$ -ALANINE
13	BENZOATE
14	BENZYLAMINE
15	BETAINE
16	BUTYRATE
17	CELLULASE
18	CITRATE
19	CREATINE
20	D-CELLOBIOSE
21	D-FUCOSE

Table 1 continued ...

Test number	Test
22	D-GALACTOSE
23	D-GLUCOSE
24	D-MELIBIOSE
25	D-TREHALOSE
26	D-XYLOSE
27	D-( $-$ )-TARTRATE
28	D-ALANINE
29	D-FRUCTOSE
30	D-GALACTURONATE
31	GLUCONATE
32	D-GLUCURONATE
33	D-MANDELATE
34	D-MANNITOL
35	D-MANNOSE
36	D-RIBOSE
37	D-SACCHARATE
38	D-SORBITOL
39	DL- $\alpha$ -AMINO-BUTYRATE
40	DL-ASPARTATE
41	DL- $\beta$ -HYDROXYBUTYRATE
42	DL-GLYCERATE
43	DL-KYNURENATE
44	DL-LACTATE

Table 1 Continued ...

Test number	Test
45	DL-MALATE
46	DL-NORLEUCINE
47	ERYTHRITOL
48	ETHANOLAMINE
49	ETHANOL
50	FERMENTATION
51	FORMATE
52	FUMERATE
53	$\gamma$ -AMINOBUTYRATE
54	GYCEROL
55	GLYCINE
56	GLYCOLATE
57	GLYCOCHOLATE
58	HEPTANOATE
59	HIPPURINATE
60	INOSITOL
61	INULIN
62	ISOBUTYRATE
63	ISOPROPANOL
64	ISOVALERATE
65	ITACONATE

Table I continued ...

Test number	Test
66	KYNURENATE
67	L-ARABINOSE
68	L-MANDELATE
69	L-(+)-TARTRATE
70	L-ALANINE
71	DL-CITRULLINE
72	L-GLUTAMATE
73	L-HISTIDINE
74	L-ISOLEUCINE
75	L-LEUCINE
76	L-LYSINE
77	L-MALATE
78	L-PHENYLALANINE
79	DL-ORNITHINE
80	L-PROLINE
81	L-SERINE
82	L-THEORINE
83	L-TRYPTOPHAN
84	LACTOSE
85	LAMARINASE

Table 1 continued ...

Test number	Test
86	MALONATE
87	M-HYDROXYBENZOATE
88	MALTOSE
89	MELEATE
90	MESO-TARTRATE
91	MUCATE
92	N-ACETYL-D-GLUCONATE
93	N-BUTANOL
94	NIACINAMIDE
95	NICOTINATE
96	PELARGONATE
97	OXALATE
98	P-AMINOBENZOATE
99	P-HYDROBENZOATE
100	PTHALATE
101	PIMELATE
102	PROPANOL
103	PROPRIONATE
104	PUTRESCINE
105	PYRUVATE
106	QUINATE

Table 1 continued ...

Test number	Test
-------------	------

107	SALICIN
-----	---------

108	SARCOSINE
-----	-----------

109	AMYLASE
-----	---------

110	SUBERATE
-----	----------

111	SEBACATE
-----	----------

112	SUCCINATE
-----	-----------

113	SUCROSE
-----	---------

114	LIPASE
-----	--------

**Table 2:** Data matrix for fermentative OTU.

OTU	TEST #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
LDC75		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
LD9555		-	-	-	-	-	+	-	-	-	-	-	-	-	-	
17802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
29570		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
33466		+	-	-	-	-	-	-	-	-	+	-	-	-	-	
LD2588		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
LD9012		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
LD9067		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
7744		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
29985		+	-	-	-	-	-	-	-	-	-	-	-	-	-	
25916		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
25917		+	-	-	-	-	-	-	-	-	-	-	-	-	-	
25919		+	-	-	-	-	-	-	-	-	+	-	-	-	-	
25920		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
27043		-	-	-	-	-	+	-	-	-	-	-	-	-	-	
LD9013		+	-	-	-	-	-	-	-	-	+	-	-	-	-	
E14048		-	-	-	-	-	+	-	-	-	-	-	-	-	-	
LD9578		+	-	-	-	-	+	-	-	-	-	-	-	-	-	
E19264		+	-	-	-	-	-	-	-	-	+	-	-	-	-	
17749		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
33125		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
LD7588		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
7		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
8		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
9		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
10		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
12		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
13		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
14		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
15		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
16		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
17		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
18		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
19		-	-	-	-	-	-	-	-	-	+	-	-	-	-	
20		-	-	-	-	-	-	-	-	-	+	-	-	-	-	

Table 2 (continued...)

OTU	TEST #														
#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
21	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
28	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
31	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
32	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
36	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
37	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
38	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-
67	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-
71	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
79	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
81	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
84	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
85	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+
94	-	+	-	-	-	+	-	-	-	+	+	+	+	-	+
95	-	+	-	-	-	+	-	-	-	+	+	+	+	-	+
97	-	+	-	-	-	+	-	-	-	+	+	+	-	-	-
99	-	+	-	-	-	+	-	-	-	+	+	+	-	-	-
100	-	+	-	-	-	+	-	-	-	+	+	+	-	-	-
101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
111	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-
112	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
113	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
BRN18	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+

**Table 2 (continued...)**

Table 2 (continued...)

Table 2 (continued...)

Table 2 (continued...)

OTU #	TEST #														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
BRN21	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-
BRN44	-	-	+	-	+	-	+	+	-	+	-	-	+	+	-
BRN93	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-
BRN99	+	-	+	-	-	-	-	+	-	-	-	-	*	-	+
BRN115	-	-	+	-	-	-	+	+	-	+	-	-	+	+	+

**Table 2 (continued...)**

Table 2 (continued...)

OTU	TEST #														
#	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
21	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+
22	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+
23	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+
24	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+
25	-	-	-	+	+	+	-	+	-	+	-	-	-	-	+
26	-	-	-	+	+	+	-	+	-	+	-	-	-	-	-
27	-	-	-	+	+	+	-	+	-	+	-	-	-	-	+
28	-	-	-	+	+	+	-	+	-	+	-	-	-	-	+
31	+	-	-	+	+	+	-	+	-	-	-	-	-	-	+
32	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
36	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+
37	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-
38	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
66	+	-	-	+	-	+	-	+	-	-	+	-	-	-	+
67	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+
71	+	-	-	+	-	+	-	+	-	-	+	-	-	-	+
79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	-	+	-	+	-	-	-	+	-	-	+	-	-	-	+
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
84	+	+	-	+	-	+	-	+	-	-	+	-	-	-	+
85	+	+	-	+	-	+	-	+	-	-	+	-	-	-	+
86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
94	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
95	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
97	-	-	-	+	-	+	-	+	-	-	+	-	-	-	+
99	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
100	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
101	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
111	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+
112	-	-	-	-	+	+	+	+	+	-	+	-	-	-	-
113	-	-	-	-	+	+	+	+	-	+	-	+	-	-	-
BRN18	+	-	-	+	+	+	+	-	-	-	+	+	+	-	+

Table 2 (continued...)

OTU	TEST #														
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
BRN21	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
BRN44	+	-	-	+	+	+	-	-	-	+	-	-	+	+	+
BRN93	-	-	-	4	-	-	-	-	-	+	-	+	+	+	+
BRN99	+	+	-	+	-	-	+	-	-	+	+	+	-	+	+
BRN115	+	+	-	+	-	+	+	-	-	+	+	+	-	+	+

Table 2 (continued..?)

Table 2 (continued...)

OTU	TEST #													
	46	47	48	49	50	51	52	53	54	55	56	57	58	59
21	-	-	-	-	+	-	+	-	+	-	-	-	-	-
22	-	-	-	-	+	-	-	-	-	-	-	-	-	-
23	-	-	-	-	+	-	+	-	-	-	-	-	-	-
24	-	-	-	-	+	-	+	-	+	-	-	-	-	-
25	-	-	-	-	+	-	+	-	+	-	-	-	-	-
26	-	+	-	-	+	-	+	-	+	-	-	-	+	-
27	-	-	-	-	+	-	+	-	+	-	-	-	+	-
28	-	-	-	-	+	-	+	-	+	-	-	-	+	-
31	-	-	-	-	+	-	+	-	+	-	-	-	-	-
32	-	-	-	-	+	-	+	-	+	-	-	-	-	-
36	-	-	-	-	+	-	+	-	+	-	-	-	-	-
37	-	-	-	-	+	-	+	-	+	-	-	-	-	-
38	-	-	-	-	+	-	+	-	+	-	-	-	-	-
59	-	-	-	-	+	-	+	-	+	-	-	-	-	-
66	-	-	-	-	+	-	+	-	+	-	-	-	+	-
67	-	-	-	-	+	-	+	-	+	-	-	-	+	-
71	-	+	-	-	+	-	+	-	+	-	-	-	+	-
79	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	-	+	-	-	-	-	-	-	-	-	-	-	+	-
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-
84	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	-	-	-	-	-	-	-	-	-	-	-	-	-	-
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-
95	-	-	-	-	-	-	-	-	-	-	-	-	-	-
97	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
101	-	-	-	-	-	-	-	-	-	-	-	-	-	-
111	-	-	-	-	-	-	-	-	-	-	-	-	-	-
112	-	-	-	-	-	-	-	-	-	-	-	-	-	-
113	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BRN18	+	-	-	-	+	-	+	-	+	-	-	-	+	-

Table 2 (continued...)

OTU	TEST													
	46	47	48	49	50	51	52	53	54	55	56	57	58	59
BRN21	-	-	-	-	+	-	+	-	-	-	-	-	-	-
BRN44	-	-	-	-	+	-	+	-	+	+	-	-	-	-
BRN93	-	-	-	-	+	-	+	+	-	-	-	-	-	-
BRN99	-	-	-	+	+	-	+	+	+	+	-	-	-	-
BRN115	-	-	-	-	+	-	+	+	+	-	-	+	-	+

Table 2 (continued...)

OTU	TEST #														
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
LDC75	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-
LD9555	+	-	-	-	-	-	+	-	-	+	+	+	+	-	-
17802	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
29570	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-
33466	+	-	-	-	-	-	+	-	-	+	+	+	+	-	-
LD2588	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LD9012	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LD9067	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
7744	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
29985	+	+	-	+	-	-	+	-	-	-	-	+	-	-	-
25916	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
25917	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
25919	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
25920	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
27043	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-
LD9013	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
E14048	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-
LD9578	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-
E19264	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-
17749	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LD7588	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
8	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
9	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
10	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
12	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
13	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
14	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
15	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
16	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
17	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
18	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
19	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
20	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-

Table 2 (continued...)

Table 2 (continued.)

OTU	TEST #													
	61	62	63	64	65	66	67	68	69	70	71	72	73	74
BRN21	-	-	-	-	-	-	-	-	+	+	-	-	+	-
BRN44	-	-	-	-	-	-	-	-	-	-	+	-	-	-
BRN93	-	-	-	-	-	-	-	-	-	-	+	-	-	-
BRN99	-	+	-	-	-	-	-	-	-	+	-	+	-	-
BRN115	+	-	-	-	-	-	+	-	-	+	-	+	+	+

**Table 2** (continued...)

Table 2 (continued...)

Table 2 (continued.)

OTU	TEST #														
	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
BRN21	-	+	-	-	-	-	-	-	+	+	-	-	+	-	
BRN44	-	+	-	-	+	+	+	-	-	+	-	-	+	-	
BRN93	-	+	-	-	-	-	-	-	-	-	-	-	+	-	
BRN99	-	+	-	+	+	+	-	-	-	-	-	-	+	-	
BRN115	-	+	-	+	+	+	+	+	-	-	-	-	-	-	

Table 2 (continued...)

Table 2 (continued...)

Table 2 (continued . . .)

OTU	TEST #													
	91	92	93	94	95	96	97	98	99	100	101	102	103	104
BRN21	-	-	-	-	-	+	-	-	-	-	-	+	-	+
BRN44	-	+	-	+	-	-	-	-	-	-	-	+	-	+
BRN93	-	+	-	-	-	-	-	-	-	-	-	+	-	+
BRN99	-	+	+	-	-	-	-	-	-	-	+	+	-	+
BRN115	+	+	-	-	-	+	-	+	-	-	-	+	-	+

Table 2 (continued...)

OTU	TEST #								
	106	107	108	109	110	111	112	113	114
LDC75	-	+	-	-	-	-	+	+	-
LD9555	+	+	-	+	-	+	+	+	+
17802	-	-	-	+	-	+	+	+	-
29570	-	+	-	+	-	+	+	+	+
33466	-	+	-	-	-	+	+	+	-
LD2588	-	-	-	-	-	-	-	-	-
LD9012	-	+	-	+	-	-	-	-	-
LD9067	-	-	-	+	-	-	-	-	-
7744	-	-	-	-	-	-	-	-	-
29985	-	-	-	-	-	-	-	-	-
25916	-	-	-	-	-	-	+	+	+
25917	-	+	-	-	-	-	+	+	-
25919	-	+	-	-	-	-	+	+	+
25920	-	-	-	+	+	-	+	+	+
27043	-	-	+	-	-	-	+	+	+
LD9013	-	-	-	-	-	-	-	-	-
E14048	-	-	+	-	-	-	+	+	+
LD9578	-	-	-	-	+	-	-	-	-
E19264	-	-	-	-	-	-	+	+	+
17749	-	-	-	-	-	-	-	-	-
33125	-	-	-	-	-	-	-	-	-
LD7588	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	+	-	-
3	-	-	-	-	-	-	+	-	-
4	-	-	-	-	-	-	+	+	+
5	-	-	-	-	-	-	+	+	+
6	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	+	-	-
8	-	-	-	-	-	-	+	-	-
9	-	-	-	-	-	-	+	-	-
10	-	-	-	-	-	-	+	-	-
12	-	-	-	-	-	-	+	-	-
13	-	-	-	-	-	-	+	-	-
14	-	-	-	-	-	-	+	-	-
15	-	-	-	-	-	-	+	-	-
16	-	-	-	-	-	-	+	-	-
17	-	-	-	-	-	-	+	-	-
18	-	-	-	-	-	-	+	-	-
19	-	-	-	-	-	-	+	-	-
20	-	-	-	-	-	-	+	-	-

Table 2 (continued...)

OTU	TEST.								
	106	107	108	109	110	111	112	113	114
21	-	+	-	-	-	+	+	-	+
22	-	-	-	-	-	+	-	+	+
23	-	-	-	-	-	+	+	+	+
24	-	-	-	-	-	+	+	-	+
25	-	-	-	-	-	+	+	-	-
26	-	-	-	-	-	+	+	-	-
27	-	-	-	-	-	+	+	-	+
28	-	-	-	-	-	+	+	-	+
31	-	-	-	-	-	+	+	-	-
32	-	-	-	-	-	+	-	+	-
36	-	-	-	-	-	+	-	+	-
37	-	-	-	-	-	+	-	+	-
38	-	-	-	-	-	+	-	+	-
59	-	-	-	-	-	+	-	+	-
66	-	-	-	-	-	+	-	+	-
67	-	-	-	-	-	+	-	+	-
71	-	-	-	-	-	+	-	+	-
79	-	-	-	-	-	+	-	+	-
80	-	-	-	-	-	+	-	+	-
81	-	-	-	-	-	+	-	+	-
83	-	-	-	-	-	+	-	+	-
84	-	-	-	-	-	+	-	+	-
85	-	-	-	-	-	+	-	+	-
86	-	-	-	-	-	+	-	+	-
87	-	-	-	-	-	+	-	+	-
88	-	-	-	-	-	+	-	+	-
89	-	-	-	-	-	+	-	+	-
90	-	-	-	-	-	+	-	+	-
92	-	-	-	-	-	+	-	+	-
93	-	-	-	-	-	+	-	+	-
94	-	-	-	-	-	+	-	+	-
95	-	-	-	-	-	+	-	+	-
97	-	-	-	-	-	+	-	+	-
99	-	-	-	-	-	+	-	+	-
100	-	-	-	-	-	+	-	+	-
101	-	-	-	-	-	+	-	+	-
111	-	-	-	-	-	+	-	+	-
112	-	-	-	-	-	+	-	+	-
113	-	-	-	-	-	+	-	+	-
BRN18	-	-	-	-	-	+	-	+	-

Table 2 (continued...)

OTU #	TEST #								
	106	107	108	109	110	111	112	113	114
BRN21	-	-	-	-	-	+	+	+	
BRN44	-	-	-	-	-	+	-	+	
BRN93	-	-	-	-	-	+	+	+	
BRN99	-	-	-	-	-	+	+	-	+
BRN115	+	-	+	-	-	+	-	-	

Table 3: Data matrix for non-fermentative OTU.

OTU #	TEST #														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
14393	-	-	-	-	+	+	-	-	-	+	-	+	+	+	+
27135	-	-	-	-	+	+	-	-	-	+	-	+	-	-	+
27120	+	-	-	-	-	+	-	-	-	+	-	+	-	-	+
27128	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-
8017	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33492	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29986	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+
27130	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
27133	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
27121	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
39	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
41	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
42	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
43	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
47	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
51	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
52	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
53	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
58	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
61	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
62	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

Table 3 (continued...)

OTU #	TEST #														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
63	-	-	-	-	-	+	-	-	+	+	-	+	+	-	+
64	-	-	-	-	-	+	-	+	+	+	-	+	-	-	+
65	-	-	-	-	-	+	-	-	+	+	-	+	+	-	+
68	-	-	-	-	-	+	-	-	+	+	-	+	-	+	+
69	-	-	-	-	-	+	-	-	+	+	-	+	-	+	+
70	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
72	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+
73	-	*	-	-	-	+	-	-	+	+	+	-	+	+	+
74	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+
75	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+
76	-	-	-	-	-	+	-	-	+	+	+	+	-	-	+
77	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-
78	-	+	-	-	-	+	-	-	+	+	+	-	-	-	+
82	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-
107	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-
108	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
109	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
110	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+
114	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+
115	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+
BRN67	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-
BRN117	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
BRN121	-	-	-	-	-	+	-	+	-	-	+	-	-	-	+
BRN133	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-

Table 3 (continued...)

Table 3 (continued...)

Table 3 (continued...)

OTU #	TEST #														
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
14393	+	-	-	+	+	+	-	+	-	+	+	-	+	+	+
27135	-	-	-	+	-	+	-	-	-	+	+	-	+	+	+
27120	-	+	-	+	+	-	-	-	-	+	-	-	+	+	+
27128	+	-	-	+	+	-	-	+	-	+	-	-	+	+	+
8017	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
33492	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29988	+	-	-	+	+	+	-	+	-	+	-	-	+	+	+
27130	+	-	-	+	+	-	+	-	-	+	-	-	+	+	+
27133	+	-	+	-	+	+	+	+	-	+	-	-	+	+	+
27121	-	-	-	+	+	-	+	-	-	+	-	-	+	+	+
2	-	-	-	-	+	-	+	+	-	+	-	-	+	+	+
11	-	-	-	-	-	+	-	-	-	+	-	-	+	+	+
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	+	+	-	+	+	-	+	-	-	+	+	+
34	+	-	-	+	+	-	+	+	-	+	-	-	+	-	-
35	+	-	-	+	-	+	-	+	-	+	-	-	+	+	+
39	-	-	-	-	+	-	+	-	-	+	-	-	+	+	+
40	-	-	-	-	-	+	+	-	-	+	-	-	+	+	+
41	-	-	-	+	+	-	+	+	-	+	-	-	+	+	+
42	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
43	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-
44	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
45	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-
46	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
47	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
48	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
49	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
51	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
52	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
53	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-
54	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
55	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
56	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
57	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
58	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
60	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-
61	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-
62	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-

Table 3 (continued...)

OTU	TEST #														
#	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
63	+	+	+	+	+	+	+	-	+	-	+	-	t	-	
64	+	+	+	+	+	+	+	-	+	+	+	-	+	+	
65	+	+	-	+	+	+	+	-	+	+	+	-	+	+	
68	+	-	+	+	-	+	-	+	+	+	+	-	+	+	
69	+	-	-	+	-	+	-	+	-	-	+	-	+	+	
70	-	-	-	+	-	-	-	-	-	-	+	-	+	-	
72	+	+	-	+	-	+	+	-	+	-	+	-	+	+	
73	+	+	-	+	+	+	+	-	+	-	+	-	+	+	
74	+	-	-	+	+	+	+	-	+	-	+	-	+	+	
75	+	-	-	+	-	+	-	+	-	-	+	-	+	+	
76	+	+	-	+	+	+	+	-	+	-	+	-	+	+	
77	+	+	-	+	+	+	+	-	+	-	+	-	+	+	
78	+	+	-	+	+	+	+	-	+	-	+	-	+	+	
82	-	-	-	+	-	-	-	-	-	-	+	-	+	+	
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
106	-	-	+	+	-	+	+	-	+	-	+	-	+	-	
107	-	-	-	-	-	+	-	+	-	+	-	+	-	+	
108	+	-	+	+	-	-	-	+	-	+	-	+	-	+	
109	-	+	+	-	-	+	+	-	+	-	+	-	+	-	
110	-	+	+	+	-	+	+	-	+	-	+	-	+	-	
114	-	+	+	+	-	+	+	-	+	-	+	-	+	-	
115	-	-	+	-	-	+	+	-	+	-	+	-	+	-	
BRN67	-	-	-	-	-	-	-	+	-	+	-	+	-	-	
BRN117	+	+	-	+	+	+	+	-	-	+	+	-	+	+	
BRN121	+	+	-	+	-	-	-	+	-	+	+	-	+	+	
BRN133	+	-	-	-	-	-	-	-	-	+	+	-	-	-	

Table 3 (continued...)

Table 3 (continued...)

OTU	TEST														
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
63	-	+	-	+	-	+	+	+	+	+	-	+	+	+	
64	-	+	-	+	-	+	+	+	+	+	-	+	+	+	
65	-	+	-	+	-	+	+	+	+	+	-	+	+	+	
68	+	+	+	+	-	+	+	+	+	+	-	+	+	+	
69	-	-	-	+	-	+	+	+	+	-	+	+	+	+	
70	-	-	-	+	-	-	+	-	-	-	-	-	-	+	
72	-	-	-	+	-	-	+	+	+	-	+	+	+	+	
73	-	+	-	-	-	+	+	+	+	-	+	+	+	-	
74	-	+	-	+	-	+	+	+	+	-	-	+	+	+	
75	-	-	-	+	-	-	+	+	+	-	+	+	+	-	
76	-	-	+	-	-	-	+	+	+	-	+	+	+	+	
77	-	-	+	+	-	-	+	+	+	-	+	+	+	+	
78	-	-	+	-	-	-	+	+	+	-	+	+	+	+	
82	-	-	-	-	-	-	+	+	-	-	-	-	-	-	
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
106	-	-	+	+	-	-	-	+	+	-	-	+	+	+	
107	-	-	+	+	-	-	-	+	+	-	-	+	+	-	
108	-	-	+	+	-	-	-	+	-	-	-	-	-	-	
109	-	-	+	+	-	-	-	+	-	-	-	-	-	-	
110	-	-	+	+	-	-	-	+	-	-	-	-	-	-	
114	-	-	+	+	-	-	-	+	-	-	-	-	-	-	
115	-	-	+	+	-	-	-	+	-	-	-	+	-	-	
BRN67	-	-	-	-	-	-	-	+	+	-	-	+	-	-	
BRN117	-	-	-	-	-	-	-	+	+	-	-	+	-	-	
BRN121	-	-	-	-	-	-	-	+	+	-	-	+	-	-	
BRN133	-	-	-	-	-	-	-	+	+	-	-	+	-	-	

Table 3 (continued...)

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OTU #	TEST #														
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
14393	+	+	-	+	-	+	+	+	-	+	+	+	+	-	-
27135	+	-	-	-	-	-	+	+	-	+	+	+	+	-	-
27120	+	+	-	-	-	-	+	+	-	-	-	+	+	-	-
27128	+	-	-	-	-	-	+	+	-	-	-	+	-	-	-
8017	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
33492	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
29988	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-
27130	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-
27133	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-
27121	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
11	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-
33	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
34	+	+	-	+	-	-	-	+	-	-	-	+	+	-	-
35	-	+	+	+	-	-	-	+	-	-	-	+	+	-	-
39	-	+	+	+	-	-	-	-	-	-	-	+	+	-	-
40	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-
41	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
42	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-
43	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-
44	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
45	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-
47	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
48	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-
49	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
50	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-
51	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-
52	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-
53	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
54	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-
55	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-
56	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-
57	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-
58	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
60	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-
61	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-
62	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-

Table 3 (continued...)

OTU #	TEST #														
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
63	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+
64	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+
65	-	+	-	-	+	-	+	-	+	+	+	+	+	-	+
68	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+
69	-	+	-	+	-	+	-	-	-	+	-	+	+	-	+
70	-	-	-	-	-	-	+	-	-	+	-	+	-	-	+
72	-	+	-	-	-	-	+	-	-	+	+	+	+	+	+
73	-	+	-	+	-	-	+	-	-	+	-	+	+	+	+
74	-	-	-	-	-	-	+	-	-	+	-	+	+	-	+
75	-	+	+	+	-	-	-	-	-	+	-	+	-	+	+
76	-	+	-	+	+	-	+	-	-	+	-	+	-	+	+
77	-	+	+	+	-	+	+	-	-	+	-	+	+	+	+
78	-	-	+	+	-	+	-	-	-	+	-	+	+	+	+
82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
107	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
108	-	-	-	-	-	+	+	+	-	-	-	-	+	-	+
109	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+
110	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-
114	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
115	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-
BRN67	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-
BRN117	-	-	-	-	-	+	-	+	-	-	-	+	+	-	+
BRN121	-	+	-	+	-	-	-	-	-	-	-	+	+	-	+
BRN133	-	+	-	+	+	-	-	-	-	-	-	+	+	-	+

Table 3 (continued...)

OTU #	TEST #														
	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
14393	+	+	+	+	+	-	-	-	+	-	-	+	+	-	
27135	+	+	-	+	+	-	+	-	-	-	-	-	-	-	
27120	-	+	-	+	+	-	+	-	-	-	-	-	-	-	
27128	-	+	-	-	+	-	-	-	-	-	-	-	-	-	
8017	-	+	-	-	-	-	-	-	-	-	-	-	-	-	
33492	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
29988	-	+	+	-	+	+	+	-	-	-	-	-	+	-	
27130	-	-	+	+	+	-	-	-	-	+	-	+	+	-	
27133	-	-	+	+	-	+	-	+	-	+	-	+	+	-	
27121	-	-	+	+	+	-	+	-	-	-	-	-	-	-	
2	-	-	-	-	+	-	+	-	-	-	-	-	-	-	
11	-	-	+	+	-	+	-	-	-	-	-	-	+	-	
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
30	-	-	-	+	+	-	+	-	-	+	-	+	-	-	
33	-	-	+	+	+	-	+	-	-	+	-	+	-	-	
34	-	+	+	+	+	-	+	-	+	-	-	+	-	-	
35	-	+	+	+	+	-	+	-	-	-	-	+	-	-	
39	-	+	-	-	+	-	-	-	-	-	-	+	-	-	
40	-	-	+	-	-	+	-	-	-	-	-	+	-	-	
41	-	-	+	+	-	+	-	-	-	+	-	+	-	-	
42	-	-	+	-	-	+	-	-	-	+	-	-	-	-	
43	-	-	+	-	-	+	-	-	-	+	-	-	-	-	
44	-	-	-	-	-	+	-	-	-	+	-	+	-	-	
45	-	+	+	+	-	+	-	-	-	+	-	+	-	-	
46	-	-	+	-	-	+	-	-	-	+	-	-	+	-	
47	-	-	-	-	-	+	-	-	-	-	-	-	+	-	
48	-	-	+	-	-	+	-	-	-	+	-	-	-	-	
49	-	-	+	-	-	+	-	-	-	+	-	-	-	-	
50	-	-	+	-	-	+	-	-	-	+	-	-	-	-	
51	-	-	+	-	-	+	-	-	-	+	-	-	-	-	
52	-	-	+	-	-	+	-	-	-	+	-	-	-	-	
53	-	+	+	+	-	+	-	-	-	+	-	+	-	-	
54	-	-	+	+	+	-	+	-	-	-	-	-	-	-	
55	-	-	+	+	+	-	+	-	-	-	-	-	-	-	
56	-	-	+	+	*	-	+	-	-	-	-	-	-	-	
57	-	-	-	-	+	-	+	-	-	-	-	-	-	-	
58	-	-	-	-	-	-	+	-	-	-	-	-	-	-	
60	-	-	-	-	-	+	+	-	-	-	-	+	-	-	
61	-	-	-	-	-	+	+	-	-	-	-	+	-	-	
62	-	-	-	-	-	+	+	-	-	-	-	+	-	-	

Table 3 (continued...)

OTU #	TEST #													
	76	77	78	79	80	81	82	83	84	85	86	87	88	89
63	+	+	+	+	+	-	-	+	-	+	-	+	-	+
64	+	+	+	+	+	-	-	+	-	+	-	+	-	+
65	-	+	-	+	+	+	-	-	-	+	-	-	-	+
68	+	+	+	+	+	+	-	+	-	-	-	+	-	-
69	+	+	+	-	+	+	-	-	+	-	-	-	-	-
70	-	+	+	-	+	+	+	-	-	-	-	+	-	-
72	-	+	+	+	+	+	+	-	+	-	-	+	-	-
73	+	+	+	+	+	+	+	-	+	-	-	+	-	-
74	-	+	-	+	+	+	-	-	-	-	-	+	-	-
75	+	+	-	-	+	-	-	-	-	-	-	-	-	-
76	-	+	+	-	+	+	-	-	-	+	-	-	-	+
77	-	+	-	-	+	-	-	+	+	-	+	+	-	-
78	+	+	+	-	+	+	+	+	-	-	+	-	-	+
82	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	+	-	+	-	+	-	-	-	-	-	+	-	-	-
107	-	-	-	-	+	-	-	-	-	-	+	-	-	-
108	+	+	-	-	+	-	-	-	-	+	+	+	-	-
109	+	+	+	-	+	-	-	+	-	+	+	-	-	+
110	+	+	+	-	+	-	-	+	-	+	+	-	-	-
114	-	+	+	-	+	-	-	+	-	+	+	-	-	-
115	-	+	+	-	+	-	-	+	-	+	-	-	-	-
BRN67	-	+	-	+	+	-	-	-	-	+	-	-	-	-
BRN117	-	+	-	+	+	+	-	+	-	-	-	-	-	-
BRN121	-	+	+	+	+	+	-	+	-	-	+	-	-	-
BRN133	-	+	+	-	+	-	-	+	-	-	+	-	-	-

Table 3 (continued...)

OTU #	TEST #													
	91	92	93	94	95	96	97	98	99	100	101	102	103	104
14393	-	+	+	-	+	-	-	-	+	-	+	+	+	+
27135	-	+	-	-	-	-	-	-	-	+	+	+	+	+
27120	-	+	-	-	+	-	-	-	-	-	+	+	+	+
27128	-	+	+	-	-	-	-	-	-	-	+	-	-	+
8017	-	+	-	-	-	-	-	-	-	-	-	-	-	+
33492	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29988	-	+	-	-	-	-	-	-	-	-	-	-	-	+
27130	-	+	-	-	-	-	-	-	+	-	-	+	-	+
27133	-	+	-	-	+	-	-	-	+	-	-	+	-	+
27121	-	+	-	-	-	-	-	-	-	-	-	-	-	+
2	-	+	-	-	-	-	-	-	-	-	-	+	-	+
11	-	-	-	-	-	-	-	-	-	-	-	+	-	+
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	+	-	-	-	-	-	-	-	-	-	+	-	-	+
33	+	-	-	-	-	-	-	-	-	-	+	-	-	+
34	+	+	-	-	-	-	-	-	-	-	+	-	-	+
35	+	-	-	+	-	-	-	-	-	-	+	-	-	+
39	-	-	-	-	-	-	-	-	-	-	+	-	-	+
40	-	-	-	-	-	-	-	-	-	-	+	-	-	+
41	+	-	-	-	-	-	-	-	-	-	+	-	-	+
42	-	-	-	-	-	-	-	-	-	-	-	-	-	+
43	-	-	-	-	-	-	-	-	-	-	-	-	-	+
44	-	-	-	-	-	-	-	-	-	-	-	-	-	+
45	+	-	-	-	-	-	-	-	-	-	-	-	-	+
46	-	-	-	-	-	-	-	-	-	-	-	-	-	+
47	-	-	-	-	-	-	-	-	-	-	-	-	-	+
48	-	-	-	-	-	-	-	-	-	-	-	-	-	+
49	-	-	-	-	-	-	-	-	-	-	-	-	-	+
50	-	-	-	-	-	-	-	-	-	-	-	-	-	+
51	-	-	-	-	-	-	-	-	-	-	-	-	-	+
52	-	-	-	-	-	-	-	-	-	-	-	-	-	+
53	+	-	-	-	-	-	-	-	-	-	-	-	-	+
54	-	-	-	-	-	-	-	-	-	-	-	-	-	+
55	-	-	-	-	-	-	-	-	-	-	-	-	-	+
56	-	-	-	-	-	-	-	-	-	-	-	-	-	+
57	-	-	+	-	-	-	-	-	-	-	-	+	-	+
58	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	+	-	+	-	-	-	-	-	-	-	-	+	-	+
61	+	-	+	-	-	-	-	-	-	-	-	+	-	+
62	-	-	-	-	-	-	-	-	-	-	-	+	-	+

Table 3 (continued...)

Table 3 (continued...)

OTU #	TEST #								
	106	107	108	109	110	111	112	113	114
14393	+	-	+	-	-	-	+	+	-
27135	-	-	+	-	-	-	-	+	+
27120	-	-	-	-	-	-	-	+	+
27128	-	-	-	-	-	-	+	+	-
8017	-	-	-	-	-	-	+	+	-
33492	-	-	-	-	-	-	-	-	-
29988	-	-	-	-	-	-	+	+	-
27130	-	+	+	+	+	+	+	+	-
27133	-	+	+	+	+	+	+	+	-
27121	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	+	+	-
11	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	+	+	-
33	-	-	-	-	-	-	+	+	-
34	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	+	+	-
39	-	-	-	-	-	-	+	+	-
40	-	-	-	-	-	-	+	+	-
41	-	-	-	-	-	-	+	+	-
42	-	-	-	-	-	-	+	+	-
43	-	-	-	-	-	-	+	+	-
44	-	-	-	-	-	-	+	+	-
45	-	-	-	-	-	-	+	+	-
46	-	-	-	-	-	-	+	+	-
47	-	-	-	-	-	-	+	+	-
48	-	-	-	-	-	-	+	+	-
49	-	-	-	-	-	-	+	+	-
50	-	-	-	-	-	-	+	+	-
51	-	-	-	-	-	-	+	+	-
52	-	-	-	-	-	-	+	+	-
53	-	-	-	-	-	-	+	+	-
54	-	-	-	-	-	-	+	+	-
55	-	-	-	-	-	-	+	+	-
56	-	-	-	-	-	-	+	+	-
57	-	-	-	-	-	-	+	+	-
58	-	-	-	-	-	-	+	+	-
60	-	-	-	-	-	-	+	+	-
61	-	-	-	-	-	-	+	+	-
62	-	-	-	-	-	-	+	+	-

Table 3 (continued...)







