USE OF THE BIOLOG™ MICROSTATION SYSTEM TO CLASSIFY AND IDENTIFY VIBRIONACEAE BACTERIA FROM A SEASONALLY-COLD OCEAN

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USE OF THE BIOLOG™ MICROSTATION SYSTEM TO CLASSIFY AND IDENTIFY *VIBRIONACEAE* BACTERIA FROM A SEASONALLY-COLD OCEAN

BY

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ABSTRACT

The Biolog™ MicroStation System was evaluated for its capacity to identify marine bacteria. The strains studied were *Vibrionaceae* type cultures and strains isolated from a seasonally-cold ocean. Using the Biolog system 54% of the *Vibrionaceae* strains were correctly identified, 18% of the strains were incorrectly identified, and 28% were not assigned an identity. Part of this study was to investigate ways in which the accuracy of the system might be improved.

Marine bacteria require Na^+ for growth. Biolog protocol was altered to bring these strains to a more optimal metabolic potential during tests. It was found that 62% of strains were more metabolically active when suspended in a marine cation supplement (MCS) plus yeast (MCSpY). Twenty-four percent of the strains were more metabolically active when suspended in salts solutions containing MCS. MCS was used in the suspending salts when testing the regional strains because this solution encouraged high metabolic activity and had a comparable identification rate to the standard suspension solution which was saline.

Approximately 36% of the regional strains were assigned a name by Biolog. Based on previous knowledge of the type of metabolism of the bacteria the names assigned were probably incorrect. It was

concluded that the Biolog system was an inadequate identification system for *Vibrionaceae*. A classification procedure based on data collected by the Biolog system was not significantly different (p-value < 0.01) from a classification based on data produced from traditional or classical bacteriological tests. This indicated that the Biolog microbial identification system could be useful for identification and classification provided that the users included reference cultures in the studies and subsequently created their own data bases. A classification meeting these criteria was produced during the course of this study.

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LIST OF ABBREVIATIONS

API	Analytical Profile Index
ATCC	American Type Culture Collection
Biolog	BIOLOG MicroStation™ System
BUGM	Biolog Universal Growth Medium
ES	Biolog ES MicroPlate™ used for the identification of *Escherichia coli* and *Salmonella* species
GN	Biolog GN MicroPlate™ used for the identification of Gram-negative bacteria
GP	Biolog GP MicroPlate™ used for the identification of Gram-positive bacteria
ID	identification
MCS	marine cation supplement
MCSpY	marine cation supplement plus yeast extract
MIDI	fatty acid methyl ester analysis
MR-VP	Methyl Red - Voges Proskauer
MT	Biolog MT MicroPlate™ with nutrient base and color chemistry, to which the user adds own carbon sources
PCA	principal-component analysis
PHB	poly-β-hydroxybutyrate
TCA	trichloracetic acid
TSA	trypticase soy agar
UPGMA	unweighted pair-group method using arithmetic averages
YEP	yeast extract proteose peptone medium

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INTRODUCTION

Species of bacteria, within the family *Vibrionaceae*, can be isolated from freshwater, estuarine, and seawater environments, as well as from the alimentary tract of man and warm-blooded animals (West and Colwell, 1984). Some species are pathogenic for man while others are pathogenic for marine vertebrates and invertebrates (West and Colwell, 1984). The most widely recognized human pathogen from this group is *Vibrio cholerae* serovar 0:1, the agent responsible for epidemic or Asiatic cholera (Baumann and Schubert, 1984). Another recognized human pathogen is *V. parahaemolyticus*. It causes gastroenteritis and is contracted by eating contaminated, usually raw, seafoods (Kelly et al. 1991). *V. anguillarum* is one of the most important agents causing epizootic outbreaks in fish cultured in marine waters throughout the world (Toranzo et al. 1987). In fact, up to one third of the species belonging to the genus *Vibrio* are considered to have pathogenic potential to humans or marine life (Farmer and Hickman-Brenner, 1992).

The abundance of pathogens within the *Vibrionaceae* stresses the importance of being able to accurately differentiate and identify members of this group. An identification scheme can be devised only after that group has first been classified. That is, the group of organisms must be recognized as being different from

other groups of organisms (Kreig, 1984). A classification scheme is based on one or more characters, or on a pattern of characters, which all the members of the group have and which other groups do not have (Kreig, 1984). As Brenner has stated, "identification is the practical use of a classification scheme to isolate and distinguish desirable organisms from undesirable ones, to verify the authenticity or special properties of a culture, or to isolate and identify the causative agent of a disease" (Brenner, 1991). Identification schemes are therefore not classification schemes, although there may be a superficial similarity (Kreig, 1984).

Prokaryote taxonomy began when Ferinard Cohn (1872) grouped bacteria according to their overall morphological appearance into cocci, short rods, elongate rods, and spirals (Trüper and Schliefer, 1992). Initially, identification of a species was thus based only on a few morphologic characters as outlined in the 1901 book by F.D. Chester named *Determinative Bacteriology*. A new era in bacterial taxonomy, dominated by physiology, began with the classification system of Orla-Jensen (1909). Numerous physiological properties of bacterial cultures were determined for characterization and then used for identification (Trüper and Schliefer, 1992). Later, enzymes were studied and metabolic

pathways elucidated. Classification and identification schemes (ie. *Bergey's Manual of Determinative Bacteriology*) thus evolved with the accumulation of information to incorporate physiological, metabolic, antibiotic susceptibility, and serological characteristics.

As new information about strains develop the classification schemes and subsequently the identification schemes for these strains change. The classification of members of the group presently known as the *Vibrionaceae* has changed dramatically as the science of taxonomy developed. If one follows the taxonomy of *Vibrio* through the successive volumes of *Bergey's Manual of Determinative Bacteriology* the changes are pronounced.

In the seventh edition of *Bergey's Manual of Determinative Bacteriology* (Breed et al. 1957), the genus *Vibrio* was assigned to the family *Spirillaceae*, based primarily on the curvature of the cell wall and negative Gram reaction. The family *Vibrionaceae* was described subsequently in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974). This family included the genus *Vibrio* along with *Aeromonas*, *Plesiomonas*, *Photobacterium*, and *Lucibacterium*. An explanation of why the genus *Vibrio* was excluded from the family *Spirillaceae* is given by West and Colwell (1984) and by Martin-

Kearley (1992). The family *Vibrionaceae* was first proposed by Veron in 1965 to group genera which were, for the most part, then known to be oxidase positive and motile by means of polar flagella. The genus *Beneckea* was included as a genus of uncertain taxonomic position (Shewan and Veron, 1974), because these species were known to produce peritrichous flagella under certain conditions (Baumann et al. 1971). In *Bergey's Manual of Systematic Bacteriology*, Vol.1 (Kreig and Holt, 1984), the family *Vibrionaceae* excludes the genus *Lucibacterium*. Members of this genus were transferred to the genus *Beneckea* by Reichelt et al. (1976) because of their possession of peritrichous as well as polar flagella. It was later found that, when grown on solid medium, many species of *Vibrio* possessed peritrichous flagellation and as a result the genus *Beneckea* was abolished (Baumann et al. 1984) and all strains subsequently became members of the genus *Vibrio* (Martın-Kearley, 1992).

To identify a member of the genus *Vibrio*, using *Bergey's Manual of Systematic Bacteriology*, Vol. 1 (Kreig and Holt, 1984), 65 tests are listed to differentiate between the 20 *Vibrio* species in Table 5.58 (Baumann et al. 1984). In the ninth edition of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994) 81 determinative tests are listed to distinguish

between the 34 listed *Vibrio* species, some of which are of uncertain taxonomic position. Other identification manuals such as *The Prokaryotes*, Vol. 3 (Balows et al. 1992), and *The Manual of Clinical Microbiology* (Balows et al. 1991), list a similar set of determinative tests to identify members of this group. Forty-nine determinative tests are listed to distinguish between the 33 *Vibrio* species of Table 15 (Farmer and Hickman-Brenner, 1992) of *The Prokaryotes*, while 59 tests are listed in Table 4 of Kelly et al. (1991) in *The Manual of Clinical Microbiology* (Balows et al. 1991) to identify a member of the 12 listed clinical *Vibrio* species.

Using "classical methods", identification of the *Vibrionaceae* is a labour intensive and slow process. This has led to a transition from classical to contemporary methodologies, with the reference point changing from the traditional multistep procedures to unitary procedures with marked emphasis on standardization, speed, reproducibility, and most recently, mechanization and automation (D'Amato et al. 1981). The most evident expression of this transition is the adaption or streamlining of classical methods in the form of "miniaturized identification systems", which are commercially available (D'Amato et al. 1981). D'Amato et al. (1991) define an

identification system: "an identification system consists of a series of tests judiciously selected and formulated to identify microorganisms to a desired level, accompanied by identification schemes, often computer based and developed for each system".

The transition to miniaturized identification systems began in the 1940s and 1950s when some forward-thinking microbiologists recognized the need for more rapid tests and subsequently approached the problem through the miniaturization of conventional procedures (D'Amato et al. 1991). This led to the first generation of commercially available identification systems. These first generation systems addressed the family *Enterobacteriaceae*, which was prudent for two reasons: (1) the *Enterobacteriaceae* represent the majority of bacteria isolated from clinical material and (2) due almost entirely to the contribution of Edwards and Ewing of the Centre for Disease Control, this family was biochemically and serologically more definitively characterized than any other group of bacteria (D'Amato et al. 1981).

The initial systems included a series of "miniaturized tubes containing individual substrates, multi compartment tubes or plates with multiple substrates, and paper strips or disks impregnated with dehydrated substrates. Tests included in the

various identification systems were judiciously selected to ensure reproducibility and provide slightly more rapid results than did their classical counterparts (D'Amato et al. 1991). Reaction endpoints were reached after 18h of incubation, and identifications were based on percentage tables published by Edwards and Ewing for *Enterobacteriaceae* (D'Amato et al. 1991). The use of the tables produced by Edward and Ewing resulted in a significant flaw that ultimately led to the development of second generation systems. That is, the formulations and volumes of the test substrates, the type of reaction indicators, and the incubation period used in the commercial systems not only differed from each other but also differed from those used by Edward and Ewing to establish the identification schemes. The commercial manufacturers of the second generation systems resolved this problem by incorporation of highly developed (sophisticated) computer generated identification data bases tailored for each system. According to D'Amato et al. (1991) additional advantages of the second-generation systems included: (1) expanded identification to include additional groups of microorganisms and yeasts (D'Amato et al. 1981); (2) reaction endpoints that can be reached after 4 h of incubation; (3) mechanized or automated inoculation, test reading, and

identification; and (4) further miniaturization of test substrate containers. Not all of the systems have all of the above mentioned attributes (D'Amato et al. 1991) and the basis under which these systems are operated varies. The bacterial characteristics that are recognized by the systems to make an identification range from specific metabolic types (API and Biolog), to fatty acid methyl ester analysis (MIDI), to genetic comparisons (Amy et al. 1992) such as DNA or RNA sequences or the ability to genetically recombine (Klausner, 1988).

Because of the obvious importance of pathogenic species, the development of these systems has centred around the identification of clinical species which are of immediate importance to humans (Amy et al. 1992). Therefore, few systems are available that can quickly and reliably identify environmental isolates (Klinger et al. 1992). Biolog Inc., (Hayward, Calf.) introduced an automated system designed to identify several hundred species, or groups, of aerobic Gram-negative bacteria within 4 to 24 h (Klinger et al. 1992). The automated identification system developed by Biolog, Inc, (Hayward, Calif.) is formally referred to as Biolog MicroStation System. The data base includes environmental taxa that are not included in the data bases of other commercial systems. Because

the Biolog automated microbial identification system has been available for a relatively short time, few assessments of its accuracy and reliability are available.

The Biolog automated microbial identification system identifies bacteria on the basis of, "You are what you eat" (Bochner, 1989b). This means that an identification of a strain is based upon a carbon source utilization pattern or "breathprint". The metabolic profile or "breathprint" is obtained from a 96-well microtiter plate which contains within each well a low-concentration complex medium plus high concentrations of a particular carbon source, such as a carbohydrate, ester, or amino acid. Each of these wells also contains the redox dye tetrazolium-violet which, upon respiration of the particular carbon source, is reduced to a violet coloured form. The colour change pattern can then be interpreted by an easy to use computerized system to identify the strain.

A brief history of carbon source utilization is presented to explain how the Biolog microbial identification system developed. The Dutch microbiologist L.E. den Dooren de Jong, in 1926, was the first proponent of carbon source utilization tests (Bochner, 1990). Den Dooren de Jong tested the capacity of fourteen different bacteria to utilize several hundred carbon and nitrogen

sources by detection of turbidity changes, all of which took many years to complete. Also developed in the 1920s was the Warburg apparatus. It was used to measure respiration rates by following oxygen uptake of cells oxidizing a particular substrate. It was a forerunner of the metabolic testing technology. The Warburg apparatus, like de Jong's turbidity testing, is much too cumbersome for practical use (Bochner, 1989a).

Since this early beginning metabolic testing generally involved the colour change of a pH indicator, as a result of fermentation of various carbohydrates (Bochner, 1990). The major problem with this testing method is that many microorganisms cannot be identified based upon carbohydrate utilization because of the fact that utilization of carbon sources does not always result in a detectable pH change in the medium or because the microorganism of interest may be asaccharolytic, e.g. *Legionella* (Mauchline and Keevil, 1991).

Utilization of carbon sources invariably leads to an increase in respiration of a bacterial culture (Bochner, 1990). It is the exchange of electrons produced during a strain's respiration of a carbon source that forms the basis of the Biolog testing system (Miller and Rhoden, 1991). The Biolog system indicates this exchange of electrons by a colour change produced

by the redox dye tetrazolium violet (Miller and Rhoden, 1991). Tetrazolium, in its oxidized state, is soluble in water and appears colourless or faintly yellow in solution. Upon reduction, it is converted to a deep purple which is insoluble in water thus making the reduction essentially irreversible (Bochner and Savageau, 1977). Bochner and Savageau (1977), believed that the reduction is a result of electrons passing from the test substrate, through the enzymatic machinery of central metabolism, the electron transport chain, and ultimately on to produce a purple formazan. Regardless of its structure, virtually any chemical substrate that is oxidized by a cell will result in the formation of NADH, which donates electrons to the electron transport chain (Bochner, 1989a). Biolog Inc. has used this simple colour change chemistry, using a single redox dye, tetrazolium violet, in conjunction with 96 well microtiter plates, to develop a comprehensive carbon source utilization testing battery which can be used on a wide variety of bacteria (Bochner, 1990).

The Biolog microtiter plate contains 96 wells which allow the simultaneous testing of 95 different carbon sources along with a negative control. Each well contains a low concentration complex medium containing minerals, amino acids and vitamins

(Bochner, 1990), plus high concentrations of a particular substrate, in a dried film. Carbon sources could include; carbohydrates, esters, amino acids, carboxylic acids, amides, and peptides (Klausner, 1988).

Biolog Inc. currently manufactures four different microplates; GN, GP, ES, and MT. The GN microplate is a panel composed of 95 carbon source utilization tests optimized for the identification of Gram-negative enterics, nonfermenters, and fastidious microorganisms (Bochner, 1990). The GP microplate is designed for the identification of Gram-positive aerobic bacteria. The ES microplate is composed of carbon utilization tests designed for characterization and biotyping of *E. coli* and *Salmonella* strains (Bochner, 1990). The MT microplate contains Biolog's redox chemistry without the addition of carbon sources, which allows the user to add a set of carbon sources tailored to a particular application (Bochner, 1990).

The Biolog system has six basic steps to identification of a strain. These are given in the Biolog MicroStation System User's Manual (Biolog, 1993): (1) Specimen Preparation: the microorganism must be grown on the appropriate nutrient medium that promotes vigorous growth. This would be a TSA (Tryptic Soy Agar) based medium for use of GN plates or BUGM (Biolog Universal

Growth Medium) for GP plates. The cells must be freshly grown [how long depends on the recommended incubation time (most organisms 4-18 hours, slow growers 36-48 hours)]; (2) Material Preparation: fill sterile disposable capped tubes with 18-20 mL of sterile, normal saline (0.85% NaCl); (3) Inoculum Preparation: establish an acceptable turbidity range on the turbidimeter and adjust the inoculum density; (4) Inoculation of the Microplate: use a multichannel pipet reservoir and a 8-Channel Repeating Pipetter to fill all wells with 150 µL of inoculum (inoculation of the panel with a cell suspension activates the chemistry); (5) Incubation: incubate at the appropriate temperature for the microorganism being tested; and (6) Reading the Results: after incubation the end result is a pattern of purple wells in an 8x12 matrix. Many microorganisms give results in 4 hours and a few species become unreadable at 24 hours. Therefore, a reading should be taken at both times periods. Reactions can be read either by eye or with a microplate reader. The absorbance (i.e. colour) in each well is referenced against the negative control well. Any noticeable purple colour above that in the control well is a positive reaction, indicating that the carbon source in the well was utilized.

The "breathprint" or metabolic pattern is a record of the

carbon sources utilized by a strain during respiration (Bochner, 1990). To identify a strain the breathprint of the strain is referenced against a library of patterns that has already been established. A computer software package performs this pattern matching function by scanning the patterns in the data base and determining the closest species match (Bochner, 1990). Miller and Rhoden (1991) best describe how the computer software package operates, "The instrument calculates a similarity index for the 10 strains in the data base whose breathprint is closest to the test strain. At the 4-h reading, only an "excellent ID" (similarity index, >0.75) is acceptable. If a lower index is obtained, the user is instructed to continue the incubation for 24h. At the 24h reading, if the index is below 0.50, the instrument reports "poor ID" or "no ID". If the index is between 0.5 and 0.74 at 24h, a "good ID" is reported along with a genus and species name. "Excellent ID" is reserved for indices of > 0.75. The 10 closest matching species are printed on all reports, although only reports with an index of >0.5 are considered an acceptable genus and species identification."

The Biolog redox chemistry is universal, ie. it functions independently of the specific structure of the electron chain (Bochner 1989b) and the bacteria do not need to grow in the

assay, although they need to be metabolically active (Mauchline and Keevil, 1991). Therefore, the system's potential to quickly and easily identify microorganisms, which can be time consuming and difficult by traditional methods, is being explored. A brief review of research which has incorporated the use of the Biolog microbial identification system follows.

Environmental and clinical samples suspected of containing *Legionella spp.* usually require elaborate techniques for identification, eg. fluorescent antibodies, or DNA probes (Armon et al. 1990). Armon et al. (1990), were the first to use the Biolog system (GN plates) to identify species as well as strains of this genus with relative simplicity. Mauchline and Keevil (1991) modified the protocol described by the manufacturer of Biolog in several ways to facilitate it's use with *Legionellae*. In particular, they found that incubating the test plates in a low oxygen atmosphere or at high cell density allowed much more rapid development of positive tests, thus facilitating identification after only 24h of incubation. Mauchline and Keevil (1991) also suggested the replacement of the carbohydrate substrates with substrates that can be utilized by these bacteria, because of their asaccharolytic nature, to increase the resolving power for *Legionellaceae* (Mauchline and Keevil, 1991).

Species identification of *Aeromonas* strains based on carbon substrate oxidation profiles has also been evaluated because most commercial identification panels for Gram-negative organisms do not have the capacity to accurately identify species within this genus (Carnahan, et al. 1989). Carnahan et al. (1989) found that all 60 *Aeromonas* strains tested could unambiguously be placed in their correct taxonomic positions using the Biolog GN microplate panel.

The Biolog system has also been used to identify clinical and non-clinical genospecies of *Acinetobacter* isolated from wastewater treatment plants (Knight et al. 1993).

Wong et al. (1992) have found that the Biolog carbon source utilization method may prove to be a useful alternative to respirometry as a means to identify strains of *Brucella spp.* at the species level. This could be of significance because bacteria of this genus cause infections in agriculturally important animals and in humans (Wong et al. 1992).

An identification problem faced by "classical" clinical microbiologists was that of trying to distinguish between *Neisserea gonorrhoeae* and its close relatives *N. meningitidis* and *N. cineria*, all of which are now easily differentiated using the Biolog system (Bochner, 1989a).

Since identification of plant-pathogenic bacteria by conventional procedures is time-consuming and costly in terms of materials, the need for more rapid, reliable, and inexpensive procedures has led to the development of techniques that can be adapted for diagnostic purposes (Jones et al. 1993). This has led plant pathogen researchers to use the Biolog automated microbial identification system. Interesting applications of the Biolog system in this area of research are outlined. Chase et al. (1992) used Biolog GN microplates to characterize *Xanthomonas campestris* strains isolated from eight hosts of aroid plants, and found that the ability to oxidize different carbon sources gave a high degree of differentiation of *X. campestris* strains from the aroids, with approximately 66% of the strains tested identified accurately to their host of origin. Hartung and Cileroto (1991) were able to separate a more aggressive strain of *X. campestris*, isolated from 20 outbreaks of citrus bacterial spot disease of Florida citrus nurseries, based solely on Biolog carbon source oxidation profiles. Verniere et al. (1993) were also interested in *X. campestris* strains and found that *X. campestris* pv. *citri* strains associated with various forms of citrus bacterial canker disease, could be differentiated into several groups based on the differential utilization of L-fucose, D-galactose, and

alaninamide as tested by Biolog. Khetmalas et al. (submitted 1994) used the Biolog GN microplate to identify *Pantoea agglomerans* as a disease causing agent of the beach pea (*Lathyrus maritimus*). A leaf spot disease of Cilantro was identified as *Pseudomonas syringae* using Biolog (Cooksey et al. 1991). Biolog also assisted in the identification of this species from a leaf spot and blight of *Abelmoschus moschatus* (Brown and McCarter, 1991). Bouzar et al. (1993), studied a diverse *Agrobacterium* (causative agent of crown gall disease) soil population from a fallow field, and found that, using the Biolog system, these strains were grouped in a manner very similar to digested DNA and protein fingerprints. Jones et al. (1993) evaluated the Biolog GN microplate system as a whole for accuracy in identifying the phytopathogenic strains of the genera *Agrobacterium*, *Clavibacter*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*.

Alterthum and Ingram (1989) used the Biolog system in a study to identify an *Escherichia coli* strain which could optimize ethanol production via utilization of sugars. To test sugar utilization the EC microplates (Biolog) were used as well as MacConkey agar supplemented with 2% carbohydrate. It is important to note here that both methods were in complete agreement for the 13 sugars examined (Alterthum and Ingram,

1989).

A number of studies incorporated the use of the Biolog system in their investigations involving the remediation of environmental contaminants:

In a remediation of environmental contaminants study Lajoie et al. (1992) proposed to create a temporary niche for a host microorganism able to degrade contaminants by adding a substrate not utilizable to most indigenous species . Biolog GN microplates were used to identify isolates from soils collected from sites previously exposed to detergents or hydrocarbons (Lajoie et al. 1992).

The Biolog system (GN microplate) was used by Fredrickson et al. (1991) to examine both large and fine scale variations in the distribution and diversity of microbial populations in a given subsurface geologic formation by determining the types of organic substrates commonly metabolized by bacteria . This was carried out in order to selectively stimulate a given group of organisms *in situ* to enhance the degradation of contaminants. It is important to note here that although the manufacturer's intended application for these plates is identification of Gram-negative bacteria, they were used in that study to obtain a metabolic profile for individual isolates (Fredrickson et al. 1991).

Fulthorpe and Wyndham first published studies based on the Biolog system in 1991 and continued to do so in 1992. They studied the transfer and expression of the catabolic plasmid pBRC60 in wild bacterial recipients in freshwater ecosystem microcosms which were exposed to various pollutants. The GN microplates were used to fingerprint any colonies that showed signs of chloroaromatic degradation. The microplate substrate utilization profiles were used to generate a single-linkage cluster analysis which shows the phenotypic dissimilarities among the isolates (Fulthorpe and Wyndham, 1992). This, in turn, demonstrated that the plasmid can proliferate in microcosms, through alternate hosts that were better adapted *a priori* to conditions in the mesocosms than the original host (Fulthorpe and Wyndham, 1991). The ability of catabolic plasmids to transfer to, and be expressed in. indigenous bacteria is clearly beneficial from the point of view of remediating contaminated waters (Fulthorpe and Wyndham, 1991).

Garland and Mills (1991) used the Biolog system as a rapid, community-level method to characterize and classify heterotrophic microbial communities, by direct incubation of whole environmental samples. The colour response in wells was quantified from digitized images of plates after selected lengths

of incubation. Principal-component analysis (PCA) of the multivariate data set (95 colour responses) differentiated samples on the relatively gross scale of microbial habitat type and the finer scale of spatial gradients within habitats. It was found that samples from three different microbial habitats (soil, freshwater, and hydroponic systems) had distinctive patterns of sole carbon source utilization on the basis of PCA of transformed colour response data (Garland and Mills, 1991). Garland and Mills (1991) felt that differentiation of samples from different habitats was a useful preliminary test of the Biolog assay while the ability to distinguish between samples within similar habitats was a much more powerful test of the resolving power of the assay. Therefore, overall widespread use of this technique could provide a more robust and ecologically relevant classification of heterotrophic microbial communities than presently exists (Garland and Mills, 1991).

The Department of Energy Deep Subsurface Microbiology Program (U.S.A.) supplied funding to extend the redox technology to identify and characterize microorganisms in the environment. The program has literally unearthed a vast variety of new microorganisms, most of which cannot be readily identified by conventional technology (Bochner, 1989a). Often industrial

microbiologists isolate organisms but find it difficult or impossible to identify them using conventional technologies. The breathprint technology is beginning to provide industrial microbiologists with an alternative approach to such problems (Bochner, 1989a). Amy et al. (1992) used the Biolog system, along with several identification systems, to try to classify bacteria from water and endolithic habitats within the deep subsurface of a mined tunnel system. Venkateswaran et al. (1993) also used the Biolog system, along with traditional methods, to carry out a microbiological examination, ie. total bacterial population, species diversity, and identity of Palau Jellyfish Lake.

Sometimes just understanding the metabolic capabilities of unknown strains (Bochner, 1990) or of communities of microorganisms (Garland and Mills, 1991; Fredrickson et al. 1991), gives us information that may not have been available before. Such information may be very useful for it can lead to the characterization and subsequent description of new species.

To assess the system's ability to identify and classify marine bacteria, a number of type cultures (ATCC) of the family *Vibrionaceae* and 80 unknown regional isolates recovered from the kelp (*Alaria esculenta*) and the giant scallop (*Placopectin*

magellanicus) were used.

The main objectives of this study were:

1. to verify the authenticity of the *Vibrionaceae* type cultures
2. to determine the capacity of the Biolog system to identify *Vibrionaceae* type cultures
3. (a) to determine the effect the cultivating medium has on the accuracy of the Biolog identification
   (b) to determine the effect that changing the ion concentration has on the accuracy of Biolog identification
4. to identify regional *Vibrionaceae* strains
5. to use numerical analysis to compare classifications produced using data collected by two different methods; (a) the Biolog MicroStation System, and (b) traditional or classical bacteriological tests.

MATERIALS AND METHODS

I. Type and Reference Cultures:

Thirty-nine type, or reference cultures belonging to the family Vibrionaceae were used in this study. These strains were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland. They included three type and one reference culture of the genus *Aeromonas*, three type cultures of the genus *Listonella*, three type cultures of the genus *Photobacterium*, and two reference and 25 type cultures of the genus *Vibrio*. In this study, type and reference cultures are called by the general terms reference cultures or reference strains. These cultures are listed in Table 1.

II. Regional Strains:

Eighty Gram-negative, Na^+-requiring, fermentative bacteria isolated from Newfoundland coastal waters were included in this study. Of these strains, 24 were isolated from the giant scallop *Placopecten magellanicus* (Gmelin) (Powell, 1978), and 56 from the brown alga *Alaria esculenta* (Hollohan, 1980). Although the isolation of these strains has been described by Hollohan (1982) and elaborated upon by Hollohan et al. (1986) additional

Table 1: Type and reference cultures of *Aeromonas*, *Photobacterium*, and *Vibrio*, from the American Type Culture Collection (ATCC).

Strain	ATCC no.
Aeromonas caviae	15468
Aeromonas hydrophilia	23211*
Aeromonas salmonicida subsp. *masoucida*	27013
Aeromonas sobria	43979
Listonella anguillarum	19264
Listonella damsela	33539
Listonella pelagia	25916
Photobacterium angustum	25915
Photobacterium leiognethi	25521
Photobacterium phosphoreum	11040
Vibrio aestuarianus	35048
Vibrio alginolyticus	17749
Vibrio campbellii	25920
Vibrio carchariae	35084
Vibrio cincinnateinsis	35912
Vibrio costicola	33508
Vibrio diazotrophicus	33466
Vibrio fisheri	7744
Vibrio fluvialis	33809

Note: Table 1 continued on the next page.

Table 1. Continued.

Vibrio furnissii	35016
Vibrio gazogenes	29988
Vibrio harveyi	14126
Vibrio hollisae	33564
Vibrio logei	29985
Vibrio marinus	15381
Vibrio mediterranei	43341
Vibrio metschnikovii	7708*
Vibrio mimicus	33653
Vibrio natriegens	14048
Vibrio nereis	25917
Vibrio nigrapulchritudo	27043
Vibrio ordalii	33509
Vibrio orientalis	33934
Vibrio parahaemolyticus	17802
Vibrio proteolyticus	15338
Vibrio salmonicida	43839
Vibrio splendidus biovar II	25914*
Vibrio tubiashii	19109
Vibrio vulnificus	27562

Note: * indicates a reference culture, the remainder being type cultures.

information concerning the environmental conditions at the time of sampling is given by Martin-Kearley and Gow (1994).

In the original studies a higher number of Gram-negative, fermentative strains was isolated from *A. esculenta* than *P. magellanicus*; a greater number of strains from algae than scallops are therefore included in this study. The numbers assigned to regional strains isolated from *A. esculenta* are 1-37 and 47-65, and those assigned to *P. magellanicus* are 38-46 and 66-80.

The eighty strains isolated from alga and scallop are called regional strains or regional cultures to distinguish them from type or reference strains or cultures.

III. Maintenance Medium:

All cultures were maintained on plates or slants of yeast extract, peptone (YEP) medium. This medium contained 0.2% (w/v) yeast extract (Difco), 0.5% (w/v) proteose peptone no. 3 (Difco), 26μM $(NH_4)_2SO_4.FeSO_4.6H_2O$ (BDH Chemicals Ltd., Poole, England), and 1.2% (w/v) agar (Oxoid Technical No.3) with 75% natural seawater and 25% deionized water (Gow and Mills, 1984). The medium was sterilized using an autoclave. To maintain the cultures in

optimal condition transfers were made periodically into YEP broth medium.

IV.1. Identification by Classical Methods:

Reference strains of the genera *Vibrio* and *Photobacterium* were tested for 37 selected characters (Table 2) as outlined by Farmer and Hickman-Brenner (1992). This was done to ensure the validity of the strains, considering that some had been subcultured over several years.

All of the determinative test media were prepared with the addition of the marine cation supplement (MCS) described in Table 3. The supplement was added to enhance growth of marine bacteria.

Thick cell suspensions of the strains were prepared in 1/20 MCS and 2% (w/v) buffered peptone water (Difco). These cell suspensions were used to inoculate the test media by the addition of one to two drops of suspension to each medium.

With the exception of *V. marinus*, *V. logei*, *V. salmonicida*,

Table 2. Determinative tests for the genera *Listonella*, *Photobacterium*, and *Vibrio* as described by Farmer and Hickman-Brenner (1992) in Table 15 of *The Prokaryotes* (Balows et al. 1992).

Oxidase (Kovacs)
Nitrate-nitrite
Indole production
Voges-Proskauer
Lysine decarboxylase (2-day)
Arginine dihydrolase (2-day)
Ornithine decarboxylase (2-day)
Lysine decarboxylase (7-day)
Arginine dihydrolase (7-day)
Ornithine decarboxylase (7-day)
Motility (25°C)
D-Glucose (acid)
D-Glucose (gas)
Lactose fermentation
Sucrose fermentation
D-Mannitol fermentation
Dulcitol fermentation
Salicin fermentation
Adonitol fermentation
myo-Inositol fermentation
D-Sorbitol fermentation

Note: Table 2 is continued on the next page.

Table 2. Continued.

L-Arabinose fermentation
Raffinose fermentation
L-Rhamnose fermentation
Maltose fermentation
D-Xylose fermentation
Trehalose fermentation
Cellobiose fermentation
∝-Methyl-D-glucoside fermentation
Erythritol fermentation
Melibiose fermentation
D-Arabitol fermentation
D-Mannose fermentation
D-Galactose fermentation
D-Galacturonate fermentation
Growth; 0 mM NaCl
Growth; 300 mM NaCl

Table 3. Marine cation supplement (MCS) 1559[1].

Sodium Chloride (NaCl)	150 g
Potassium Chloride (Kcl)	3.7 g
Magnesium Chloride ($MgCl_2.6H_2O$)	51 g

[1] The formulation of this supplement was described by Farmer and Hickman-Brenner (1992). It is a modification of the original "electrolyte supplement" of Furness et al. (1978). This supplement was added in the ratio of one volume of supplement to nine volumes of medium in all of the determinative test media listed in Table 2. It was not required for the oxidase test.

and *P. phosphoreum*, which were incubated at 16-18°C, all other strains were incubated at 25°C. The former had optimal growth temperatures between 15 and 20°C while the latter grew better at 25°C. All determinative tests were incubated for 48 hours unless otherwise specified.

Of the 37 determinative tests carried out, 23 were to determine acid production from carbohydrates. Four were to determine the presence of the following internal enzymes; oxidase, arginine dihydrolase, lysine, and ornithine decarboxylase. Four others were to determine the presence of the following biochemical pathways; reduction of nitrate to nitrite, production of gas from D-glucose, the production of acetylmethylcarbinol during glucose fermentation (Voges-Proskauer), and the production of indole from the amino acid tryptophan. Motility at 25°C and Na^+ requirement were the two remaining tests which were visual tests.

IV.2. Media Preparation:

A. Carbohydrate utilization:

To determine the utilization of a carbohydrate each medium was prepared using the compound at a concentration of 1% (w/v) along with dehydrated phenol red broth base (Difco) at 1.6% (w/v)

as an indicator. The prepared media were dispensed into 15 x 125 mm tubes in 5-mL amounts, and subsequently autoclaved. Following inoculation and incubation of these tests, fermentation of the carbohydrate was determined by a color change from red to yellow.

The production of gas from the fermentation of D-Glucose was determined. This was done by examining a Durham tube for a gas bubble following fermentation of the glucose.

B. Oxidase test:

The oxidase test detects the presence of cytochrome oxidase or indophenol oxidase, which catalyses the transport of electrons to electron acceptors in the electron transport chain (Pezzlo, 1992). This determinative test was done as outlined in The Clinical Microbiology Procedures Handbook, Vol. 1 (Pezzlo, 1992).

C. Nitrate to nitrite:

To test for denitrification and the reduction of nitrate to nitrite, a broth was prepared from dehydrated nitrate medium (Difco) at 0.9% (w/v). This medium was dispensed into 15 x 125 mm culture tubes, with Durham tubes, and autoclaved. Denitrification by the reference cultures was determined using

the method described by Blazevic and Ederer (1975).

D. Indole production:

Indole is one of the degradation products of the metabolism of the amino acid tryptophan. With medium rich in tryptophan, indole can be detected by it's ability to combine with certain aldehydes to form a coloured compound (Pezzlo, 1992), in this case the aldehyhde *p*-dimethylaminobenzaldehyde (a component of Kovac's reagent).

Tubes (15 x 125 mm) containing sterile 5-mL portions of trypticase peptone (BBL) at 1% (w/v) were inoculated with the test organism. At the end of the incubation period 1.0 mL of Kovac's reagent was added to the tube to detect the presence of indole which was indicated by the development of a red color. The preparation of Kovac's reagent is described in the Clinical Microbiology Procedures Handbook (Pezzlo, 1992).

E. Voges-Proskauer test:

The Voges-Proskauer (VP) test detects the production of acetoin or acetylmethylcarbinol, an intermediate in the butylene glycol pathway of glucose fermentation (Pezzlo, 1992). To

perform this test MR-VP medium (Difco) was used. The reagents and procedure followed are outlined in the *Clinical Microbiology Procedures Handbook* (Pezzlo, 1992).

F. Decarboxylase and dihydrolase tests:

The lysine and ornithine decarboxylase and arginine dihydrolase tests were carried out as described in the Clinical Microbiology Procedures Handbook (Pezzlo, 1992), using decarboxylase base Moeller (Difco). This test detects the attack of the carboxyl portion of a specific amino acid, forming a corresponding amine product (Pezzlo, 1992). The decarboxylation of lysine and ornithine yield cadaverine and putrescine respectively, while arginine is converted to citrulline by a dihydrolase reaction (Pezzlo, 1992).

G. Motility at 25°C:

Examination by phase contrast microscopy was used to determine motility at 25°C.

H. Growth at 0 mM and 300 mM NaCl:

Testing for growth at 0 mM NaCl detects a Na^+ requirement of

a strain. If growth cannot occur at 0 mM NaCl, the strain probably has a growth requirement. The reference strains were tested for growth at 0 mM NaCl following the procedure outlined by Noble and Gow (1993) with one exception. Because relatively few strains were used, and only two Na^+ concentrations were tested, a drop of thick cell suspension was applied directly onto the plating medium instead of using the multipoint inoculator.

V. Biolog Test Procedures.

A. Cultivating the bacteria:

In order for a strain to be identified by the Biolog System it must be grown on the appropriate agar medium. In the Biolog Inc. procedures manual (1993) it is stated that the choice of the agar medium is very important because it must support growth and promote retention of full metabolic activity to accurately match the metabolic patterns in the GN data base. Biolog Inc. recommends the use of Biolog Universal Growth Medium (BUGM) or Tryptic Soy Agar (TSA) for identification of environmental strains. Because marine bacteria (including the genera *Listonella, Photobacterium* and *Vibrio*) have a highly specific need for Na^+ for growth (MacLeod, 1965), several different agar

media choices, with and without the addition of marine salts, were considered in this study. These were;

1. Tryptic Soy Agar, [Soybean-Casein Digest Agar (BBL), 4% (w/v)].
2. Tryptic Soy Agar (as above) plus glycerol (Sigma) (0.2%).
3. Tryptic Soy Agar plus glycerol (as above) with 1/10 MCS[1].
4. Biolog Universal Growth Medium, [3.7% (w/v) (Biolog, Inc.)].
5. Biolog Universal Growth Medium (as above) with 1/10 MCS.
6. Yeast Extract Proteose Peptone Medium (as described earlier), sclidified with 1.5% (w/v) Oxoid Technical Agar (No. 3).

[1]Marine Cation Supplement (Table 3)

In this study, medium No.3 (Tryptic Soy Agar plus glycerol with 1/10 MCS) was the growth medium used most extensively. The use of glycerol as an additive in this medium was recommended by B. Bochner of Biolog, Inc. (Personal Communication). All cultures were maintained on YEP medium, which was described earlier. The strains were subcultured twice on the medium of choice before they were suspended in salts solutions in preparation for inoculating the Biolog plates. This was done to ensure viability and metabolic vigour.

B. Suspending salts:

In one set of experiments each of the reference cultures was suspended in three different suspending salt solutions; 0.85% NaCl, 1/10 MCS, and 1/10 MCS plus 0.01% (w/v) yeast extract (Difco) (MCSpY). The yeast extract in the latter medium was added to provide trace organic nutrients. All three salt solutions were prepared by dispensing 20 mL of the solution into disposable new glass tubes (20 x 125 mm) which were capped and then sterilized by autoclaving.

C. Inoculum preparation:

An acceptable turbidity range was established using the Biolog turbidimeter (590 nm) in conjunction with the Biolog GN MicroPlate high and low turbidity standards. This gave an approximate cell density of about 3 x 10^8 cells/mL (Biolog, Inc.). The turbidimeter was adjusted to zero using uninoculated suspending salts. Uniform cell suspensions, within the acceptable turbidity range, were then prepared. Uniform cell suspensions were achieved by premoistening a sterile swab by dipping it in the sterile suspending salt solution and then rolling the swab over the colonies so as to not carry over any nutrients from the

agar medium. The swab was then twirled and pressed against the inside surface of the tube to break up any clumps and release the cells into the saline, and then used in a vertical stirring motion. A sterile transfer pipet was used to create a uniform suspension if this was necessary.

D. Inoculation of the MicroPlates:

The uniform cell suspension was transferred into a sterile multichannel pipet reservoir (Biolog, Inc.). An 8-channel repeating pipetter (Biolog, Inc.), fitted with sterile disposable tips (Biolog, Inc.), was used to fill all the wells of the Biolog GN MicroPlate with 150 μL of cell suspension.

E. Incubation:

Many Gram-negative strains give an adequate growth pattern after 4 to 6 hours of incubation. The pattern of a few enteric species is not readable if incubated overnight (Biolog, Inc.). Therefore, 4-6 and 24 h readings were taken. Most MicroPlates were incubated at 30°C, and 24 h readings were chosen for analysis. The exceptions were *V. marinus* and *V. salmonicida* which are psychrophilic and were incubated at 4°C for 72 h.

F. Reading the MicroPlates:

The GN MicroPlates were read using a Biolog MicroPlate reader in conjunction with MicroLog release 3 computer software. To use the automated reader the lid of the MicroPlate was removed and the plate was placed in the drawer of the reader. The reader was set at a wavelength of 590 nm and the "automatic variable threshold" option was used to obtain a printout of the "breathprint" and an identification of the strain. During the course of the study the identifications obtained via MicroStation system release 3.00 software were updated to release 3.50 specifications by reentering the "breathprints" of the strains using release 3.50 and obtaining new identifications.

VI.1. Classification according to criteria described in the ninth edition of *Bergey's Manual of Determinative Bacteriology*.

An objective of the study was to compare the classifications of *Vibrionaceae* produced using results from data collected by two different methods; (i) the Biolog MicroStation System, and (ii) traditional or classical bacteriological tests as described in *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994). A classification had been produced by Martin-Kearley (1992) and

Martin-Kearley and Gow (1994) based on criteria described by Baumann et al. (1984) in *Bergey's Manual of Systematic Bacteriology* (Kreig and Holt, 1984). Because of this early study results and procedures for most tests required to produce a classification based on Holt et al. (1994) were available in the M.Sc. thesis of Martin-Kearley (1992). The classification generated in the study presented here was based on 71 determinative tests which are listed in Table 4. Martin-Kearley (1992) and Martin-Kearley and Gow (1994) based their classification on 91 tests. Tests that were recommended by Holt et al. (1994) and that were not included in Table 4 are: presence of polar flagella; lateral flagella on solid medium; straight rods; poly-β-hydroxybutyrate (PHB) accumulation; luminescence; Na^+-requirement for growth; organic growth factor requirements; and utilization of δ-aminovalerate, L-arginine, and butyrate as sole sources of carbon and energy. Either these results were not available from the earlier work of Martin-Kearley (1992) or were characters that would not contribute to the numerical analysis. An exampleof the latter is Na^+ requirement which is a character present in most of the strains studied. Test results were not available for the exoenzymes amylase, gelatinase,

Table 4. Determinative tests based on Table 5.52A of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994) for which Martin-Kearley (1992) data were used.

Amylase	D-Galactose	D-Mannose	Swarming
Gelatinase	D-Galacturonate	Melibiose	Pigment
Lipase	D-Gluconate	L-Ornithine	Oxidase
Alginase	L-Glutamate	Pelargonate	Acetoin
Chitinase	Glutamate	L-Proline	4°C, growth
Acetate	Glutarate	Propanol	30°C, growth
Aconitate	D-Glucuronate	Propionate	35°C, growth
β-Alanine	Glycine	Putrescine	40°C, growth
D-Alanine	Heptanoate	Pyruvate	Arginine dihydralase
L-Alanine	L-Histidine	Quinate	
γ-Aminobutyrate	ρ-Hydroxybenzoate	L-Rhamnose	Nitrate reduction
L-Arabinose	β-Hydroxybutyrate	D-Ribose	
L-Aspartate	*myo*-Inositol	Salicin	D-Glucose, gas
Caprate	Isobutyrate	L-Serine	
Caproate	α-Ketoglutarate	D-Sorbitol	
Caprylate	DL-Lactate	Sucrose	
Cellobiose	Lactose	Trehalose	
Citrate	L-Leucine	L-Tyrosine	
Citrulline	DL-Malate	Valerate	
Ethanol	D-Mannitol	D-Xylose	

lipase, and alginase. These results were determined by Rhian Walther as part of an undergraduate research project and used in this study.

VI.2. The Determination of Exoenzymes.

General Procedure:

Exoenzymes were tested using buffered artificial seawater agar medium supplemented with 0.01% yeast extract, 0.01% proteose peptone # 3, and the appropriate substrate. Standard sized plates of the medium were inoculated by swabbing an area of the plate with a suspension of the test organism, adjusted to an optical density of 1.0 at 600nm using a Spectronic 100. These strains were incubated at 25°C and were tested after 48 h incubation.

A. Amylase:

To test for this enzyme 1% soluble starch (product S-9765, Sigma) was added to the medium. To detect the enzyme the plate was flooded with a 50% solution of Lugol's iodine. A positive reaction was scored if a clear zone developed around the patch of growth.

B. Lipase:

To test for this enzyme 1% polyethylene sorbiton mono-oleate (Tween-80) (product P-1754, Sigma) was added to the medium. Lipase activity was detected by the appearance of a precipitate of calcium oleate.

C. Gelatinase:

To test for this enzyme 2% Bacto Gelatin (Difco) was incorporated into the medium. To develop this test the plates were flooded with 30% trichloracetic (TCA). If a clear zone developed around a colony this was interpreted as a positive result.

D. Alginase:

To test for alginase activity an overlay technique was used. That is, a thin layer of medium, equal to 10-mL, was poured into the plate to form a base. This base did not contain alginate. After the base solidified a second medium similar to the first, except that it also contained medium viscosity alginic acid (product A-2033, Sigma) at a 1% concentration was added. Because of the nature of the medium it was necessary to add agar at a 1.6% concentration instead of the more usual 1.2% for Oxoid

Technical Agar No. 3. The extra agar was necessary to achieve adequate solidification of the overlay. The enzyme was detected by a method described, for the detection of cellulase, by Teather and Wood (1982). The plates were developed by flooding with a 1 mg/L solution of Congo red in distilled water. After 15 minutes the Congo red was discarded and the plates were flooded with a 1 M solution of NaCl for a further 15 minutes. A clear zone around the area of growth indicated a positive test.

VII. Cluster Analysis:

The object of a cluster analysis is to sort a sample of individuals under investigation into groups such that the degree of association is higher between members of the same group, and lower between members of different groups. This results in a determination of the overall similarity between the strains, and a hierarchial ordering of the strains according to their similarity (Hollohan, 1982; Martin-Kearley, 1992). In this study, clusters of similar strains are presented in dendrograms.

Two software packages were used for cluster analysis. One was the Biolog MLCLUST program used to produce dendrograms of specified user created data bases. The other was SPSS for Windows, Professional Statistics package, release 6.0 (SPSS Inc.;

Chicago, Illinois).

The Biolog MLCLUST program used a multiplicative measure of probability and the UPGMA (unweighted pair-group method using arithmetic averages) clustering method to produce dendrograms. The MLCLUST program initially produces dendrograms with relative ease but manipulation of these dendrograms, ie. removal of strains or tests, is labour intensive. This difficulty is a result of the data bases used by the MLCLUST being located in a separate Biolog program. Although the MLCLUST program was used in the initial stages, the numerical analysis presented in this study was done using the SPSS Professional Statistics package. This package allowed a user to easily enter data and manipulate dendrograms. To produce dendrograms the Ward's method of hierarchial analysis in conjunction with the squared Euclidean distance measure for binary data was used. Ward's method employs a distance, or dissimilarity coefficient which measures distances between objects, in a space defined in various ways. This method of numerical analysis is known for producing dendrograms with well-defined clusters. A detailed discussion of this method is available by Romesburg (1984).

In one set of experiments each of the reference cultures was

suspended in different salt solutions as described in Section V.B. It was anticipated that this might result in changes in the breathprint patterns. Dendrograms were produced from the breathprint data obtained (breathprint data were obtained as outlined in Section V.F. and converted to binary format; positive or variable reactions were recorded as 1, negative reactions were recorded as 0). Strain placement within the dendrograms was used as an indication of the effect of the suspending salts on the classification.

Classifications were also produced based upon data collected by two different methods; (i) by traditional or classical bacteriological tests, and (ii) by the Biolog MicroStation System. SPSS was used to perform a Chi-square analysis on the classifications to determine whether the results of one classification would predict that of the other. The actual data matrices obtained by the two methods are available in the Appendix.

RESULTS

I. Verifying the authenticity of the *Vibrionaceae* reference cultures using classical methods and the Biolog system:

To test the identification ability of the Biolog system, *Vibrionaceae* type and reference cultures were used. In a preliminary study, for which the results are not shown, approximately 40% of the strains tested could not be identified, or were incorrectly identified, by the system. For the reference cultures that were not identified, or were incorrectly identified, there were two possibilities. One was that the Biolog system could not correctly identify them. The other was that the cultures themselves were either mis-labelled or impure. The reference cultures that were correctly identified by using the Biolog system are listed in Table 5.

The next step was to identify the strains using the classical tests recommended in the determinative table of Farmer and Hickman-Brenner (1992) in *The Prokaryotes* Vol.3 (Balows et al. 1992). This was done and analyzed. Problematic cultures, based on comparisons between Biolog results and the classical test results were replaced with new cultures from the ATCC. These cultures, which are called replacement cultures, are listed in Table 6. All replacement cultures were retested using the

Table 5. Reference cultures of the genera *Listonella*, *Photobacterium*, and *Vibrio*, correctly identified by the Biolog System.

Strain
Listonella anguillarum
Listonella damsela
Listonella pelagia
Photobacterium angustum
Photobacterium leiognethi
Vibrio aestuarianus
Vibrio alginolyticus
Vibrio campbellii
Vibrio carchariae
Vibrio cincinnateinsis
Vibrio diazotrophicus
Vibrio fluvialis
Vibrio furnissii
Vibrio harveyi
Vibrio hollisae
Vibrio mediterranei
Vibrio mimicus

Note: Table 5 is continued on the next page.

Table 5. Continued.

Vibrio natriegens
Vibrio parahaemolyticus
Vibrio proteolyticus
Vibrio splendidus biovar II
Vibrio tubiashii
Vibrio vulnificus

Table 6. Replacement cultures ordered directly from the American Type Culture Collection (ATCC).

Strain	ATCC number
P. phosphoreum	25914
V. costicola	33508
V. fisheri	7744
V. fluvialis	33809
V. hollisae	33564
V. marinus	15381
V. metschnikovii	7708
V. mimicus	33653
V. natriegens	14048
V. nereis	25917
V. nigrapulchritudo	27043
V. orientalis	33934
V. proteolyticus	15338
V. salmonicida	43839
V. splendidus	25914

Biolog system. Also they were checked by retesting using the classical method recommended by Farmer and Hickman-Brenner (1992). The test results obtained, using the Farmer and Hickman-Brenner (1992) determinative table for the *Listonella*, *Photobacterium*, and *Vibrio* reference cultures are given in Tables 7 through 16. These test results are those of the original cultures that were considered pure and correctly labelled (Table 5) and of the replacement cultures (Table 6). In Tables 7 to 16 test results from *The Prokaryotes* are given in addition to the test results obtained. This was done to make comparisons of the expected test results and the results obtained in this study more convenient.

Species of *Aeromonas* were not tested using the Farmer and Hickman-Brenner (1992) classification because the determinative table used was not appropriate for this group. Also, the Biolog system gave the expected identification for *A. salmonicida* subsp. *masoucida* and the three species *A. caviae*, *A. hydrophilia*, and *A. sobria* were later shown to clustered together indicating their expected similarity.

Tables 7 through 16 are fashioned like Table 15 of Farmer and Hickman-Brenner (1992) in *The Prokaryotes*, Vol.3 (Balows et al. 1992). In the original table of Farmer and Hickman-Brenner

Table 7. Results of the determinative tests for clinical species of the genera *Listonella* and *Vibrio*; *V. alginolyticus*, *V. carchariae*, *V. cincinnatiensis*, and *L. damsela*.

Test	*V.alginolyticus*		*V.carcharia*		*V.cincinnat.*		*L.damsela*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	+	+	+	+	(+)	w+
Nitrate->nitrite	+	+	+	+	+	+	+	+
Indole production	(+)	+	+	+		-	-	-
Voges-Proskauer	+	+	-	+	-	-	+	-
Lysine decarb. (2day)	+	+	+	+	(+)	+	V	-
Arginine dihyd. (2day)	-	-	-	-	-	-	+	+
Ornithine decarb.(2day)	V	-	+	-	-	-	-	-
Lysine decarb. (7day)	+	+	+	+	+	+	V	-
Arginine dihyd. (7day)	-	-	-	-	-	-	+	+
Ornithine decarb.(7day)	V	-	+	+	-	-	-	-
Motility (25°C)	+	+	+	+	+	+	+	+
D-Glucose-acid	+	+	+	+	+	+	+	+
D-Glucose-gas	-	-	-	-	-	-	V	+
Lactose ferm.	-	+/-	-	-	-	-	-	+/-
Sucrose ferm.	+	+	+	+	+	+	(-)	+/-
D-Mannitol ferm.	+	+	+	+	+	+	-	+/-
Dulcitol ferm.	-	-	-	+/-	-	+/-	-	-
Salicin ferm.	-	-	-	+/-	+	+	-	-
Adonitol ferm.	-	-	-	+/-	-	+/-	-	-

Note: Table 7 is continued on the next page.

Table 7. Continued.

myo-Inositol ferm.	-	-	-	+/-	+	+	-	-
D-Sorbitol ferm.	-	+/-	-	+/-	-	+/-	-	-
L-Arabinose ferm.	-		-		+		-	
Raffinose ferm.	-	-	-	-	-	-	-	+/-
L-Rhamnose ferm.	-	-	-	-	-	-	-	+/-
Maltose ferm.	+	+	+	+	+	+	+	+
D-Xylose ferm.	-		-		V		-	-
Trehalose ferm.	+	+	-	+	+	+	V	-
Cellobiose ferm.	+	+	+	+	+	+	+	+
∝-Methyl-D-glucoside f.	-	+/-	-	-	V	+	(-)	-
Erythritol ferm.	-	-	-	+/-	-	-	-	-
Melibiose ferm.	(-)	-	-	+/-	-	+/-	-	+/-
D-Arabitol ferm.	-	+/-	-	+/-	-	-	-	-
D-Mannose ferm.	+	+	+	+	+	+	+	+
D-Galactose ferm.	(-)	+/-	+	+	(+)	+	+	+
D-Galacturanate ferm.	-	-	-	-	-	-	-	-
Growth; 0% NaCl	-		-		-	-	-	-

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,
symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

Table 8. Results of the determinative tests for clinical species of the genus *Vibrio*; *V. fluvialis*, *V. furnissii*, *V. hollisae*, and *V. metschnikovii*.

Test	*V.fluv-ialis*		*V.furn-issii*		*V.holl-isae*		*V.metsc-hnikovii*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	+	+	+	+	-	-
Nitrate->nitrite	+	+	+	+	+	+	-	-
Indole production	(+)		-	+	+	+	-	+
Voges-Proskauer	-	-	-	-	-	-	+	+
Lysine decarb. (2day)	-	-	-	-	-	+	+	-
Arginine dihyd. (2day)	+	+	+	+	-	-	V	-
Ornithine decarb.(2day)	-	-	-	-	-	-	-	-
Lysine decarb. (7day)	-	-	-	-	-	+	+	-
Arginine dihyd. (7day)	+	+	+	+	-	+	+	+
Ornithine decarb.(7day)	-	-	-	-	-	-	-	-
Motility (25°C)	+	+	+	+	+	+	V	+
D-Glucose-acid	+	+	+	+	+	+	+	+
D-Glucose-gas	-	-	+	+	-	-	-	-
Lactose ferm.	-	+/-	-	-	-	+/-	(-)	+
Sucrose ferm.	+	+	+	+	-	+	+	+
D-Mannitol ferm.	+		+	+	-	+	(+)	+
Dulcitol ferm.	-	+/-	-	-	-	-	-	+/-
Salicin ferm.	(+)	+	-	+/-	-	+/-	(-)	-
Adonitol ferm.	-	-	-	-	-	+/-	-	-
myo-Inositol ferm.	-	+/-	-	-	-	-	V	+

D-Sorbitol ferm.	-	+/-	-	+/-	-	+/-	V	+/-
L-Arabinose ferm.	+	+	+	+	+	+/-	-	-
Raffinose ferm.	-	+/-	-	+/-	-	+/-	-	+/-
L-Rhamnose ferm.	-	-	-	+/-	-	-	-	+/-
Maltose ferm.	+	+	+	+	-	+	+	+
D-Xylose ferm.	-	-	-		-	-	-	-
Trehalose ferm.	+	+	+	+	-	+	+	+
Cellobiose ferm.	(-)	+	-	-	-	+	-	-
∝-Methyl-D-glucoside f.	-	-	-	-	-	-	-	-
Erythritol ferm.	-		-	-	-	-	-	+/-
Melibiose ferm.	-	-	-	-	-	-	-	+/-
D-Arabitol ferm.	(+)	+	-	+/-	-	+/-	-	+/-
D-Mannose ferm.	(+)		+	+	+	+	-	+
D-Galactose ferm.	+	+	(+)	+	+	+	+	
D-Galacturanate ferm.	+	+	+	+	-	-	V	-
Growth; 0% NaCl	+		+		-		+	

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

Table 9. Results of the determinative tests for clinical species of the genera *Vibrio*; *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus*.

Test	*V. mimicus*		*V.parahaemolyticus*		*V. vulnificus*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	+	+	+	+
Nitrate->nitrite	+	+	+	+	+	+
Indole production	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-
Lysine decarb. (2day)	+	+	+	+	+	+
Arginine dihyd. (2day)	-	-	-	-	-	-
Ornithine decarb.(2day)	+	+	+	-	+	-
Lysine decarb. (7day)	+	+	+	+	+	+
Arginine dihyd. (7day)	-	-	-	-	-	-
Ornithine decarb.(7day)	+	+	+	+	+	+
Motility (25°C)	+	+	+	+	+	+
D-Glucose-acid	+	+	+	+	+	+
D-Glucose-gas	-	-	-	-	-	-
Lactose ferm.	-	+/-	-	-	-	+/-
Sucrose ferm.	-	+/-	-	+/-	(-)	+/-
D-Mannitol ferm.	(+)	+	(+)	+	(+)	-
Dulcitol ferm.	-	-	-	-	-	-
Salicin ferm.	-	-	-	+	-	+

Table 9. Continued.

Adonitol ferm.	-	+/-	-	-	-	-
myo-Inositol ferm.	-	-	-	+/-	-	-
D-Sorbitol ferm.	-	+/-	-	-	-	-
L-Arabinose ferm.	-	-	+		-	
Raffinose ferm.	-	+/-	-	-	-	+/-
L-Rhamnose ferm.	-	+/-	-	-	-	-
Maltose ferm.	+	+	+	+	+	+
D-Xylose ferm.	-	-	-		-	
Trehalose ferm.	+	+	+	+	+	+
Cellobiose ferm.	-	-	-	+	+	+
∝-Methyl-D-glucoside f.	-	+/-	-	-	-	-
Erythritol ferm.	-	+/-	-	-	-	-
Melibiose ferm.	-	+/-	-	+/-	-	+/-
D-Arabitol ferm.	-	+/-	-	-	-	+/-
D-Mannose ferm.	-	+	+	+	+	+
D-Galactose ferm.	+	+	+	+	+	+
D-Galacturanate ferm.	V	+/-	-	-	-	-
Growth; 0% NaCl	+	+ 60h	-	+ 3d	-	

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -
h hours
d days

Table 10. Results of the determinative tests for non-clinical species of the genera *Listonella* and *Vibrio*; *V. aestuarianus*, *L. anguillarum*, *V.campbellii*, and *V. costicola*.

Test	*V. aest-uarianus*		*L. ang-uillarum*		*V. camp-bellii*		*V. cost-icola*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	+	+	+	+	+	+
Nitrate->nitrite	+	+	+	+	+	+	V	+
Indole production	+	+	(+)	+	(+)	+	-	-
Voges-Proskauer	-	-	+	+	-	-	(+)	-
Lysine decarb. (2day)	V	-	-	-	+	+	-	-
Arginine dihyd. (2day)	+	+	(+)	+	-	-	V	+
Ornithine decarb.(2day)	-	-	-	-	(-)	-	-	-
Lysine decarb. (7day)	(+)	-	-	-	+	+	-	-
Arginine dihyd. (7day)	+	+	+	+	-	-	V	+
Ornithine decarb.(7day)	-	-	-	-	(-)	-	-	-
Motility (25°C)	+	+	+	+	+	+	(+)	+
D-Glucose-acid	+	+	+	+	+	+	+	+
D-Glucose-gas	-	-	-	-	-	-	-	-
Lactose ferm.	(+)	+/-	-	-	-	-	-	-
Sucrose ferm.	+	+	+	+	-	-	V	+
D-Mannitol ferm.	+	+	+	+	V	+	V	-
Dulcitol ferm.	-	-	-	-	-	+/-	-	-
Salicin ferm.	-	-	-	-	-	+	-	-
Adonitol ferm.	-	+/-	-	-	-	-	-	-
myo-Inositol ferm.	-	+/-	-	-	-			

Table 10. Continued.

D-Sorbitol ferm.	-	-	+	+	-	-	-	-
L-Arabinose ferm.	-	+/-	(+)	+	-		-	
Raffinose ferm.	-	-	-	-	-	+/-	-	-
L-Rhamnose ferm.	-	-	-	+/-	-	+/-	-	-
Maltose ferm.	+	+	+	+	+	+	(+)	+
D-Xylose ferm.	-	+/-	-	-	-		-	-
Trehalose ferm.	+	+	+	+	+	+	(+)	+
Cellobiose ferm.	+	+	(+)	+	+	+	(-)	+
∝-Methyl-D-glucoside f.	-	-	-	-	-	-	V	-
Erythritol ferm.	-	-	-	-	-	-	-	-
Melibiose ferm.	(-)	+	-	+/-	-	-	-	-
D-Arabitol ferm.	(-)	-	-	-	-	+/-	-	-
D-Mannose ferm.	+	+	+	+	+	+	(+)	+
D-Galactose ferm.	+	-	V	+/-	-	-	(-)	+
D-Galacturanate ferm.	-	-	-	-	-	-	-	-
Growth; 0% NaCl	-	-	-		-		-	

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symnols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

Table 11. Results of the determinative tests for non-clinical species of the genus *Vibrio*; *V. diazotrophicus*, *V. fisheri*, *V. gazogenes*, and *V. harveyi*.

Test	*V. diazotrophicus*		*V. fisheri*		*V. gazogenes*		*V. harveyi*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	+	+	-	-	+	+
Nitrate->nitrite	+	+	(+)	+	-	-	+	+
Indole production	+	+	-	+	-	-	+	+
Voges-Proskauer	-	-	-	+	V	+	-	-
Lysine decarb. (2day)	-	-	V	+	-	-	+	+
Arginine dihyd. (2day)	-	-	-	-	-	-	-	-
Ornithine decarb.(2day)	-	-	-	-	-	-	(+)	-
Lysine decarb. (7day)	-	-	+	+	-	-	+	+
Arginine dihyd. (7day)	+	+	-	-	-	-	-	-
Ornithine decarb.(7day)	-	-	-	-	-	-	+	+
Motility (25°C)	+	+	+	+	+	+	+	+
D-Glucose-acid	+	+	(+)	+	+	+	+	+
D-Glucose-gas	-	-	-	-	+	+	-	-
Lactose ferm.	V	+/-	(-)	-	(+)	+/-	-	+/-
Sucrose ferm.	+	+	-	+	+	+	+	+
D-Mannitol ferm.	+	+	V	+	+	+	+	+
Dulcitol ferm.	-	+/-	-	-	-	-	-	+/-
Salicin ferm.	+	+	(+)	+/-	+	+	-	+
Adonitol ferm.	-	+/-	-	-	-	-	-	+/-

Table 11. Continued.

myo-Inositol ferm.	-	+/-	-	-	-	-	-	+/-
D-Sorbitol ferm.	-	+/-	-	-	V	-	-	+/-
L-Arabinose ferm.	+	+	-		+		-	+
Raffinose ferm.	-	+/-	-	-	-	-	-	+
L-Rhamnose ferm.	-	+	-	-	-	+/-	-	+/-
Maltose ferm.	+	+	+	+	+	+	+	+
D-Xylose ferm.	+	+	-		+		-	+
Trehalose ferm.	+	+	-	+	+	+	+	+
Cellobiose ferm.	+	+	+	+	+	+	+	+
∝-Methyl-D-glucoside f.	-	-	-	-	-	-	-	-
Erythritol ferm.	-	+/-	-	-	-	-	-	+/-
Melibiose ferm.	-	+/-	(+)	-	V	+	V	+
D-Arabitol ferm.	-	+/-	-	-	-	-	-	+/-
D-Mannose ferm.	(+)	+	+	+	+	+	+	+
D-Galactose ferm.	+	+	+	+	(+)	+	+	+
D-Galacturanate ferm.	+	-	-	-	-	-	-	-
Growth; 0% NaCl	-	-	-		-	-	-	-

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

Table 12. Results of the determinative tests for non-clinical species of the genus *Vibrio*; *V. logei*, *V. mediterranei*, *V. marinus*, and *V. natriegens*.

Test	*V. logei* (15°C)		*V. mediterranei*		*V. marinus* (15°C)		*V. natriegens*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	+	+	+	+	+	-/w
Nitrate->nitrite	+	+	+	+	+	+	+	+ g
Indole production	-	-	+	+	-	-	-	-
Voges-Proskauer	-	-	-	-	-	-	-	-
Lysine decarb. (2day)	+	+	V	+	-	-	-	-
Arginine dihyd. (2day)	-	-	V	-	-	-	-	-
Ornithine decarb.(2day)	V	-	-	-	-	-	-	-
Lysine decarb. (7day)	+	+	V	+	+	+	-	+
Arginine dihyd. (7day)	-	-	+	-	-	-	-	-
Ornithine decarb.(7day)	(+)	-	-	-	-	-	-	-
Motility (25°C)	+		+	+	-	+	+	+
D-Glucose-acid	+	+	+	+	+	+	+	+/-
D-Glucose-gas	-	-	-	-	-	-	-	-
Lactose ferm.	(-)	+/-	-	+	-	-	-	-
Sucrose ferm.	(-)	+	+	+	-	-	+	-
D-Mannitol ferm.	(+)	-	+	+	-	-	+	-
Dulcitol ferm.	-	+/-	-	-	-	+/-	-	-
Salicin ferm.	-	-	-	+	-	+/-	+	-
Adonitol ferm.	-	-	-	-	-	-	-	-
myo-Inositol ferm.	-	-	-	+/-	-	-	-	-

Table 12. Continued.

D-Sorbitol ferm.	-	-	+	+	-	-	-	-
L-Arabinose ferm.	-		-		-		+	
Raffinose ferm.	-	-	-	+/-	-	-	(+)	-
L-Rhamnose ferm.	-	-	-	-	-	-	+	-
Maltose ferm.	+	+	+	+	+	+	+	-
D-Xylose ferm.	-		-		-		-	
Trehalose ferm.	V	+	+	+	-	+/-	+	-
Cellobiose ferm.	+	+	+	+	-	-	(+)	-
∝-Methyl-D-glucoside f.	-	-	-	-	-	-	(+)	-
Erythritol ferm.	-	-	-	-	-	+/-	-	-
Melibiose ferm.	-	+/-	V	+	-	+/-	(+)	-
D-Arabitol ferm.	-	-	-	-	-	-	+	-
D-Mannose ferm.	+	+	+	+	-	+	(+)	-
D-Galactose ferm.	+	+	+	+	+	+/-	V	-
D-Galacturanate ferm.	-	-	-	-	-	-	-	-
Growth; 0% NaCl	-	-	-	-	-		-	-

[1] expected results as listed in Table 15 given bt Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

Table 13. Results of the determinative tests for clinical species of the genus *Vibrio*; *V. nereis*, *V. nigrapulchritudo*, *V. ordalii*, and *V. orientalis*.

Test	*V.nereis*		*V.nigri-pulch-ritudo*		*V.ordal-ii*		*V.orien-talis*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	+	+	+	+	+	+
Nitrate->nitrite	+	+	+	+	V	-	+	+
Indole production	V	+	+	+	-	-	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-
Lysine decarb. (2day)	-	-	-	-	-	-	+	+
Arginine dihyd. (2day)	(+)	+	-	-	-	-	-	-
Ornithine decarb. (2day)	-	-	-	-	-	-	-	-
Lysine decarb. (7day)	-	-	-	-	-	-	+	+
Arginine dihyd. (7day)	+	+	-	-	-	-	V	+
Ornithine decarb. (7day)	-	-	-	-	-	-	-	-
Motility (25°C)	+	+	+	+	+	+	+	+
D-Glucose-acid	+	+	+	+	+	+	+	+
D-Glucose-gas	-	-	-	-	-	-	-	-
Lactose ferm.	-	-	-	+/-	-	+	-	+/-
Sucrose ferm.	+	+	-	-	+	-	+	+
D-Mannitol ferm.	-		-	-	-	-	-	+
Dulcitol ferm.	-	-	-	-	-	-	-	-
Salicin ferm.	-	-	-	+/-	-	-	-	+/-
Adonitol ferm.	-	-	-	+/-	-	-	-	+/-

Table 13. Continued.

myo-Inositol ferm.	-	-	-	-	-	-	-	-
D-Sorbitol ferm.	-	-	-	-	-	-	-	+/-
L-Arabinose ferm.	-	+/-	-	-	-		-	+/-
Raffinose ferm.	-	-	-	-	-	-	-	+/-
L-Rhamnose ferm.	-	-	-	-	-	-	-	-
Maltose ferm.	+	+	+	+	+	+	+	+
D-Xylose ferm.	-	-	-	+/-	-		-	-
Trehalose ferm.	+	+	+	+	-	-	+	+
Cellobiose ferm.	-	-	+	+	-	-	+	+
∝-Methyl-D-glucoside f.	-	+/-	-	-	-	-	-	-
Erythritol ferm.	-		-	-	-	-	-	-
Melibiose ferm.	-	-	-	-	-	-	-	-
D-Arabitol ferm.	-	+/-	-	-	-	-	-	+/-
D-Mannose ferm.	+	+	V	+	-	-	+	+
D-Galactose ferm.	-	+/-	V	+	-	-	-	+
D-Galacturanate ferm.	-	-	-	-	-	-	-	-
Growth; 0% NaCl	-		-		-		-	

[1] expected results as listed in Table 15 given by Farmer, and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

Table 14. Results of the determinative tests for the non-clinical species of the genera *Listonella* and *Vibrio*; *L. pelagia*, *V. proteolyticus*, and *V. salmonicida*.

Test	*L. pelagia* biogroup 1&2			*V. proteolyticus*		*V. salmonicida* (15°C)	
	Exp[1] 1	Exp[1] 2	Obs[2]	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	+	+	-/w	+	+	+	+
Nitrate->nitrite	+	+	+	+	+	-	+
Indole production	-	+	-	+	+	-	-
Voges-Proskauer	-	-	-	-	-	-	-
Lysine decarb. (2day)	-	-	-	+	+	-	+
Arginine dihyd. (2day)	-	-	-	+	+	-	-
Ornithine decarb.(2day)	-	-	-	-	-	-	-
Lysine decarb. (7day)	-	-	-	+	+	-	-
Arginine dihyd. (7day)	-	-	-	+	+	-	-
Ornithine decarb.(7day)	-	-	-	-	-	-	-
Motility (25°C)	+	+	+	+	+	-	+
D-Glucose-acid	+	+	+	+	+	V	-
D-Glucose-gas	-	-	-	-	-	-	-
Lactose ferm.	-	-	-	-	+/-	-	-
Sucrose ferm.	(-)	-	+	-	+/-	-	-
D-Mannitol ferm.	+	V	+	+		+	-
Dulcitol ferm.	+	+	-	-	-	-	-
Salicin ferm.	-	-	-	-	-	-	-

Table 14. Continued.

Adonitol ferm.	- -	+/-	-	+/-	-	+
myo-Inositol ferm.	- -	-	-	+/-	-	+/-
D-Sorbitol ferm.	- -	-	+	+	-	-
L-Arabinose ferm.	- -		-	+/-	-	
Raffinose ferm.	- -	-	-	+/-	-	-
L-Rhamnose ferm.	- -	+	-	-	-	-
Maltose ferm.	+ +	+	+	+	-	-
D-Xylose ferm.	- -		-	+/-	-	
Trehalose ferm.	+ +	+	+	+	+	+
Cellobiose ferm.	(-)-	+	+	+	-	-
∝-Methyl-D-glucoside f.	- -	-	-	-	-	-
Erythritol ferm.	- -	-	-		-	-
Melibiose ferm.	V -	-	-	+/-	-	-
D-Arabitol ferm.	- -	-	-	+/-	-	-
D-Mannose ferm.	(+)+	+	+	+	-	-
D-Galactose ferm.	(+)V	+	-	+/-	+	+
D-Galacturanate ferm.	- -	-	-	-	-	+/-
Growth; 0% NaCl	- -	-	-		-	-

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive change
- no visual change
+/- difficult to interpret as + or -

Table 15. Results of the determinative tests for non-clinical species of the genus *Vibrio*; *V. splendidus*, *V. tubiashii*, and *V. vulnificus*.

Test	*V. splendidus* biogroup (1&2)		*V. tubiashii*		*V. vulnificus* biogroup (2)	
	Exp[1] 1 2	Obs[2]	Exp	Obs	Exp 2	Obs
Oxidase (Kovacs)	+ +	+	+	+	+	+
Nitrate->nitrite	+ +	+	+	+	+	+
Indole production	+ +	+	+	+	-	+
Voges-Proskauer	- -	-	-	-	-	-
Lysine decarb. (2day)	- -	-	-	-	+	+
Arginine dihyd. (2day)	(-) -	-	(+)	+	-	-
Ornithine decarb.(2day)	- -	-	-	-	-	-
Lysine decarb. (7day)	- -	-	-	-	+	+
Arginine dihyd. (7day)	(+) V	-	+	+	-	-
Ornithine decarb.(7day)	- -	-	-	-	-	+
Motility (25°C)	+ +	+	+	+	+	+
D-Glucose-acid	+ +	+	+	+	+	+
D-Glucose-gas	- -	-	-	-	-	-
Lactose ferm.	- -	+/-	-	-	V	+/-
Sucrose ferm.	(+) -	+	+	+	-	+/-
D-Mannitol ferm.	+ +	+	+	+	-	-
Dulcitol ferm.	- -	+/-	-	-	-	-

Table 15. Continued.

Salicin ferm.	-	-	+/-	-	+/-	-	+
Adonitol ferm.	-	-	+/-	-	-	-	-
myo-Inositol ferm.	-	-	+/-	-	-	-	
D-Sorbitol ferm.	-	-	+/-	-	-	-	-
L-Arabinose ferm.	-	-	+/-	-		-	
Raffinose ferm.	-	-	+/-	-	-	-	+/-
L-Rhamnose ferm.	-	-	+/-	-	-	-	-
Maltose ferm.	+	+	+	+	+	+	+
D-Xylose ferm.	-	-	+/-	-		-	
Trehalose ferm.	+	+	+	+	+	+	+
Cellobiose ferm.	+	+	+	(+)	+	+	+
∝-Methyl-D-glucoside f.	-	-	-	-	-	-	-
Erythritol ferm.	-	-	+/-	-	-	-	-
Melibiose ferm.	-	-	+/-	(-)	+	-	+/-
D-Arabitol ferm.	-	-		-	-	-	+/-
D-Mannose ferm.	+	-	+	+	+	V	+
D-Galactose ferm.	-	-	+/-	V	+	+	+
D-Galacturanate ferm.	-	-	-	-	-	-	-
Growth; 0% NaCl	-	-		-		-	

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

Table 16. Results of the determinative tests for species of the genus *Photobacterium; P. angustum, P. leiognathi, and P. phosphoreum.*

Test	*P.angus-tum*		*P.leiog-nathi*		*P.phos-phoreum*	
	Exp[1]	Obs[2]	Exp	Obs	Exp	Obs
Oxidase (Kovacs)	V	-	V	-	(-)	-
Nitrate->nitrite	V	-	(+)	+	(+)	+
Indole production	-	-	-	-	-	-
Voges-Proskauer	V	-	(+)	-	+	+
Lysine decarb. (2day)	V	-	V	-	(+)	-
Argine dihyro. (2day)	+	-	(+)	+	+	-
Ornithine decarb.(2day)	-	-	-	-	-	-
Lysine decarb. (7day)	V	-	V	-	(+)	-
Arginine dihyd. (7day)	+	+	(+)	+	+	-
Ornithine decarb.(7day)	-	-	-	-	-	-
Motility (25°C)	+	+	+	+	V	+
D-Glucose-acid	+	+	+	+	+	+
D-Glucose-gas	-	-	V	-	-	+
Lactose ferm.	-	+/-	-	+/-	-	+/-
Sucrose ferm.	V	+	-	+/-	-	-
D-Mannitol ferm.	-	+/-	-	-	-	-
Dulcitol ferm.	-	-	-	-	-	-
Salicin ferm.	-	-	-	-	-	-
Adonitol ferm.	-	-	-	-	-	-

Table 16. Continued.

myo-Inositol ferm.	-	-	-	-	-	-
D-Sorbitol ferm.	-	-	-	+/-	-	-
L-Arabinose ferm.	-		-		-	
Raffinose ferm.	-	-	-	+/-	-	-
L-Rhamnose ferm.	-	-	-	+/-	-	-
Maltose ferm.	V	+	-	-	+	+
D-Xylose ferm.	+	+	-	-	-	+/-
Trehalose ferm.	V	-	-	-	-	-
Cellobiose ferm.	-	+/-	-	+/-	-	+/-
∝-Methyl-D-glucoside f.	-		-	-	-	-
Erythritol ferm.	-	-	-	-	-	-
Melibiose ferm.	-	-	-	+/-	-	-
D-Arabitol ferm.	-	-	-	-	-	-
D-Mannose ferm.	+	+	+	+	+	+
D-Galactose ferm.	-	+	(+)	+	+	+
D-Galacturanate ferm.	-	-	-	-	-	-
Growth; 0% NaCl	-		-		-	

[1] expected results as listed in Table 15 given by Farmer and Hickman-Brenner (1992).

[2] observed results from this study,

symbols: + definite positive visual change
- no visual change
+/- difficult to interpret as + or -

(1992) the genus *Vibrio* was divided into clinical and non-clinical species and the species of the genus *Photobacterium* were assigned to their own group. The symbols used by Farmer and Hickman-Brenner (1992) are: +, strains are 90% to 100% positive; (+), 75% to 89.9% positive; V, 25.1% to 74.9% positive; (-), 10.1% to 25% positive; -, 0 to 10% positive. The meaning of the symbols used in this study to record results are given as footnotes on Tables 7 through 16.

Table 7 gives the results of the determinative tests of the clinical species *V. alginolyticus*, *V. carchariae*, *V. cincinnatiensis*, and *L. damsela*. It can be seen from this table that the observed results for *V. alginolyticus* and *V. cincinnatiensis* are in very close agreement with the expected results and if the difficult to interpret tests, indicated by the +/-, are considered negative they are in very good if not perfect agreement. This indicated that the +/- results could be considered negative (-) and they were from this point on. On the other hand, both *V. carchariae* and *L. damsela* had different than expected results for the Voges-Proskauer test. Also, *V. carchariae* was negative for the ornithine decarboxylase 2-day test which should have been positive. However, the 7-day reaction was positive as expected. This may indicate a

difficulty in reading the test done at the shorter incubation period. This strain also gave a positive result for trehalose fermentation which was not expected. Because the four of these strains were correctly identified by the Biolog System (Table 5), it was concluded that these cultures were both pure and correctly labelled.

The results of the determinative tests for the clinical *Vibrio* species *V. fluvialis*, *V. furnissii*, *V. hollisae*, and *V. metschnikovii* are given in Table 8. The observed results for *V. fluvialis* are very close to those expected while *V. furnissii* gave an unexpected test result for Indole production. *V. hollisae* on the other hand differed from the expected for the following tests; lysine decarboxylase (2 and 7-day), arginine dihydrolase (7-day), sucrose fermentation, D-mannitol fermentation, maltose fermentation, trehalose fermentation, and cellobiose fermentation. Although these results were a source of concern the Biolog system correctly identified this species as well as the two others. *V. metschnikovii* is not included in the Biolog data base and it's validity could not be ensured by this system. This species gave incorrect results for the classical tests; indole production, lysine decarboxylase (2 and 7-day), and D-mannose fermentation. Verification of this strain could thus

be problematic if this strain was not a direct replacement culture obtained from the ATCC.

Table 9 lists the determinative test results for three more clinical *Vibrio* species. These are *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus*. Three of these species were correctly identified by Biolog (Table 5) and little problem was indicated by the determinative test results of Farmer and Hickman-Brenner (1992). *V. mimicus* differed from the expected results for only D-mannose fermentation while *V. parahaemolyticus* and *V. vulnificus* differed from the expected for ornithine decarboxylase (2-day) production and salicin fermentation. *V. parahaemolyticus* also tested positively for cellobiose fermentation when the listed classical test result is negative.

Table 10 gives the determinative test results for the following non-clinical species; *V. aestuarianus*, *L. anguillarum*, *V. campbellii*, and *V. costicola*. The observed test results for *L. anguillarum* and *V. costicola* were very closely matched to the expected test results and these cultures were considered to be pure and correctly labelled. *V. aestuarianus* and *V. campbellii* differed from the expected results by one test each. These were D-galactose fermentation and salicin fermentation, respectively. Both of these cultures, along with *V. anguillarum*, were correctly

identified by Biolog (Table 5) and were considered to be pure and correctly labelled.

In Table 11 the test results of the determinative tests are given for the non-clinical species *V. diazotrophicus*, *V.fisheri*, *V.gazogenes*, and *V. harveyi*. From this table it can be seen that the test results for *V. gazogenes* were as expected. *Vibrio diazotrophicus* differed in respect to L-rhamnose and D-galacturanate fermentation but was correctly identified using the Biolog system (Table 5). *V. harveyi* was also correctly identified by Biolog (Table 5) but showed four inconsistencies with the expected Farmer and Hickman-Brenner (1992) results. These were salicin and raffinose fermentation, as well as L-arabinose and D-xylose fermentation. *V. fisheri* also disagreed in four tests. These were indole production, the Voges-Proskauer test, sucrose fermentation, and trehalose fermentation. Because this species is not included in the Biolog data base its purity could not be assured in this way. Due to difficulty in maintaining this culture it was already a replacement culture at the time of testing (Table 6). Although it had given some inconsistent results it's identity was ensured.

V. logei, *V. mediterranei*, *V. marinus*, and *V.natriegens* are the non-clinical species for which the determinative test results

are shown in Table 12. Of these species the identification of *V. mediterranei* and *V. natriegens* was verified by Biolog (Table 4) even though *V. mediterranei* differed from the expected classical determinative test results by 3 tests [arginine dihydrolase (7-day), lactose fermentation, and salicin fermentation] and *V. natriegens* by eight [lysine decarboxylase (7-day), sucrose, D-mannitol, salicin, L-rhamnose, maltose, trehalose, and D-arabitol fermentation]. *V. logei* and *V. marinus* were not included in the Biolog data base and therefore their identity could not be verified using the Biolog system. The determinative test results did not indicate a problem with *V. logei* with the test results being very similar to those expected. *V. marinus* differed by two tests; motility at 25°C (which may have been a result of the above optimal incubation temperatures), and fermentation of D-mannose which, on its own, did not indicate a problem.

Table 13 lists the determinative test results for *V. nereis*, *V. nigrapulchritudo*, *V. ordalii*, and *V. orientalis*. None of these species is included in the Biolog data base and therefore could not be checked in this manner. None of these species showed classical test results that were in disagreement with the expected results. Both *V. ordalii* and *V. orientalis* differed by just two fermentative tests each. These were lactose and sucrose

for *V. ordalii* and D-mannitol and D-galactose for *V. orientalis*. It was concluded that the classical test results for both *V. nereis* and *V. nigrapulchritudo* were in very good agreement with the expected test results.

V. proteolyticus, another non-clinical species, gave determinative test results in very close agreement with those expected (Table 14). *L. pelagia* (Table 14), also correctly identified by Biolog (Table 5), gave inconsistent test results for two tests, fermentation of dulcitol and L-rhamnose for biogroup 1. These cultures were considered to be pure and correctly labelled. *V. salmonicida* (Table 14) is not included in the Biolog data base and it differed from the expected by five tests. These were nitrate to nitrite, lysine decarboxylase (2-day), motility (25°C), D-mannitol fermentation, and adonitol fermentation. Although motility at this temperature may be explained by the technique used to detect it, the reason for the positive reactions for the other three tests is unknown. Because the tests were done on a new replacement culture from the ATCC the culture was considered to be pure and correctly labelled.

V. splendidus, *V. tubiashii*, and *V. vulnificus* of Table 15 were all correctly identified by Biolog (Table 4). *V. tubiashii* also had classical determinative test results in very close

agreement with those expected. The test results observed for *V. splendidus* were in perfect agreement with those expected for biogroup 1 although the strain is supposed to belong to biogroup 2 (Table 1), in which case the positive reactions for sucrose and D-mannose fermentation were not expected. Indole production, ornithine decarboxylase production (7-day), and salicin fermentation gave positive reactions where negative results were expected for the non-clinical species of *V. vulnificus*. If this strain is the clinical one (Table 9), as is suspected, then disagreement lies only in the ornithine decarboxylase (2-day) and salicin fermentation tests.

Table 16 lists the determinative test results for members of the genus *Photobacterium*. From this table it can be seen that the results for *P. leiognathi* were very close to what was expected for the determinative tests, therefore validity is ensured. *P. angustum* was negative for arginine dihydrolase (2-day) production when it was expected to be positive and was positive for D-galactose fermentation when the expected result was negative. The difference of two test results was not considered to show contamination or mislabelling. This species was correctly identified by the Biolog System (Table 5). Although *P. phosphoreum* gave three contradictory determinative

test results [arginine dihydrolase production (2 and 7-day), and D-glucose-gas] the purity of this culture is almost certain as it was a new replacement culture ordered from the ATCC (Table 6).

II. Effect of changing Biolog test procedures on the capacity of the Biolog system to accurately identify and promote metabolic activity of *Vibrionaceae* reference strains.

A. Effect of media used for cultivation:

V. proteolyticus, *V. diazotrophicus*, and *V. metschnikovii* were cultivated on several growth media to determine if the culturing medium would have any effect on the subsequent identification given by the Biolog system. Table 17 gives a summary of the test results. *V. proteolyticus* showed the greatest similarity level when it was grown on TSA plus glycerol in 1/10 MCS at 0.875 and 0.904 when repeated. The two media that were made from Biolog's Universal Growth Medium resulted in high identification similarity levels (0.828). The identification of the culture grown on TSA plus glycerol was very good at a similarity of 0.794. When this species was grown on YEP medium its identification by Biolog was below the acceptable level and when grown on TSA alone it was not identified at all. These results indicate that the TSA plus glycerol in 1/10 MCS give the best identification by Biolog for this species. They also

Table 17. Biolog similarity level at which *V. proteolyticus*, *V. diazotrophicus*, and *V. metschnikovii* were identified by the Biolog system when grown on different media (note; no ID = no identification by Biolog).

Media	Strain		
	V. proteo-lyticus	*V. diazotr-ophicus*	*V. metsch-nikovii*
Tryptic Soy Agar (TSA)	no ID	0.514	no ID
TSA plus glycerol	0.794	0.759	no ID
TSA plus glycerol with 1/10 MCS	0.875	0.386	no ID
TSA plus glycerol with 1/10 MCS (replicate)	0.904	0.671	no ID
Biolog Universal Growth Medium (BUGM)	0.828	0.886	no ID
BUGM with 1/10 MCS	0.828	0.733	no ID
Yeast Extract Proteose Peptone	0.492	0.450	no ID

indicate that BUGM or simply the TSA plus glycerol would have given acceptable identifications also. The results of *V. diazotrophicus* were quite different. In this case BUGM gave the highest identification similarity level at 0.886, TSA plus glycerol was second at 0.757 and was followed by BUGM with MCS at 0.733. The results of growing this species on TSA plus glycerol with MCS were not reliable as this species was not identified at a acceptable level on this medium in one case (0.386) while a Biolog similarity of 0.671 was obtained when it was replicated. This species was also close to borderline with a similarity of only 0.514 when grown on TSA, while it was not identified at a significant level when grown on YEP. *V. metschnikovii*, on the other hand, was not identified by Biolog when grown on any of the media. From the results presented it was decided that either TSA plus glycerol with MCS or BUGM would be suitable for cultivating the bacteria. From the results for *V. metschnikovii* it was apparent that, in some instances, the failure to obtain an identification could not be avoided by changing the growth media. The results for *V. diazotrophicus* showed that both acceptable identifications and unacceptable identifications could be obtained with the same medium. It would appear that BUGM would be a good choice of medium but it is a medium for which the

formula is not known by the user and there is only one supplier. It was decided to use TSA plus glycerol with 1/10 MCS as the cultivation medium because it did give good results and can be made in the laboratory. Although the data are not presented here, it was a general observation that this medium consistently gave profuse growth for all of the strains tested.

B. Effect of ions in suspending:

Each of the reference strains used in this study was tested in three different suspending salts solutions. Names and similarity levels were determined for the strains, by using the Biolog software to read and interpret the breathprints obtained from bacteria suspended in salt solution. The name assigned by Biolog was only deemed acceptable if the similarity level was 0.500 or greater. `The calculated value of "similarity" in the MicroLog software is used as a calling criterion to judge the reliability and confidence of the identification (Biolog manual, 1993).

According to the Biolog manual, many marine bacteria prefer a higher level of salt and will give more positive reactions in the MicroPlate if cultured on a more preferred medium (eg. Marine Agar) or suspended in 2% to 5% NaCl. Therefore, if one is

interested in characterizing the metabolism of marine bacteria under conditions that are more optimal Biolog recommends comparing procedures for preparing inocula. Because the MicroLog data base was created following standard procedures, that is probably by growing the cultures on an appropriate medium and suspending them in 0.85% NaCl, we would expect the similarity level of the identifications of the reference cultures to be greatest for strains suspended in the 0.85% NaCl solution. On the other hand, the other suspending solutions with higher ion content may have been more optimal for these marine bacteria and therefore the number of positive wells in the MicroPlate may be greater. Of the genera tested, *Aeromonas* does not have a Na^+ requirement and therefore the additional salts may not affect strains belonging to this genus.

Each of the strains listed in Table 1 was treated with three suspending salts solutions. These were 0.85% NaCl, marine cation supplement (MCS), and marine cation supplement plus yeast extract (MCSpY). When the results of the Biolog 95 carbon source tests for the three suspending salt treatments were analysed by numerical methods the tests for which a borderline result was scored by the Biolog platereader were converted to a positive result for analysis. The MicroLog software was used to assign a

name and similarity level to the strains. The results of these trials are given in Tables 18-24.

The results of the MicroLog identifications of the *Aeromonas* strains tested are given in Table 18. From this table it can be seen that only the strain *A. salmonicida* ss. *masoucida* was identified correctly and this happened using all three suspending salts. It is also apparent from this table that three of the species had the greatest number of positive wells when they were suspended in MCSpY. For the other species the breathprint was most extensive in just MCS. In Figure 1 it is shown that most of the *Aeromonas* strains suspended in the three salt solutions clustered together in cluster f. *A. salmonicida* and *A. caviae* in the three salt solutions, and *A. hydrophilia* and *A. sobria* suspended in 0.85% NaCl and MCS were directly aligned. The *A. sobria* suspended in MCS plus yeast extract was found in cluster (h) and *A. hydrophilia*, also suspended in MCSpY, was found to be distantly related in cluster d. This is probably because of the large difference in the number of positive wells found in this treatment (MCSpY) compared to the other two treatments. It was concluded that good identifications were not obtained for species of *Aeromonas* except *A. salmonicida*. For *A. salmonicida* either 0.85% NaCl or MCS were suitable suspending salts.

Figure 1. Dendrogram showing numerical analysis of *Vibrionaceae* reference cultures suspended in 0.85% NaCl, MCS (*), and MCSpY (**).

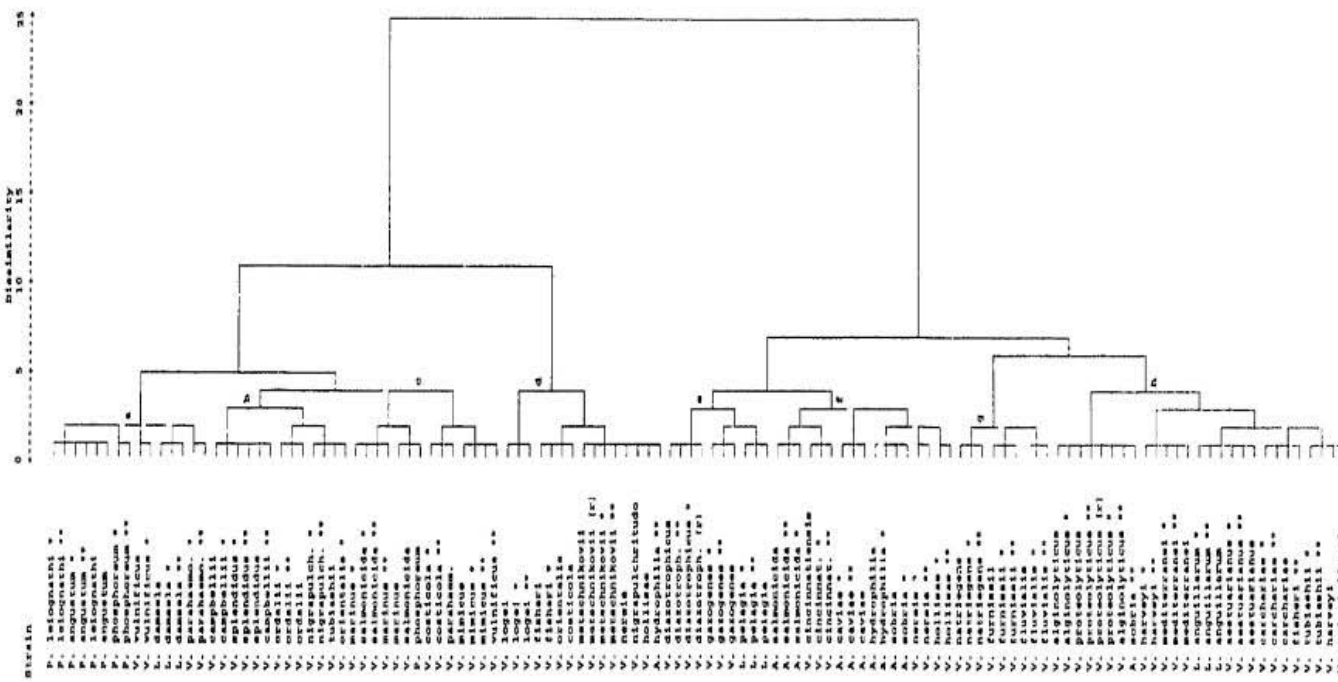

Dissimilarity
0
5
10
15
20
25
Strain
A
B
C
D
E
F
G

Table 18. Name assigned by Biolog, Biolog similarity level, and number of positive wells in the MicroPlate for *Aeromonas* strains suspended in different salt solutions.

Strain[1]	Name assigned by Biolog (similarity >0.500)	Similarity	wells positive
A. caviae	*Aer.* med-1k DNA 5A	0.609	45
A. caviae *	no identification	0.239	50
A. caviae **	no identification	0.465	52
A. hydrophilia	*V. cholerae*	0.696	37
A. hydrophilia *	no identification	-	40
A. hydrophilia **	*Pasteurella caballi*	0.557	18
A. salmonicida	*A. s.* ss *masoucida*	0.786	40
A. salmonicida *	*A. s.* ss *masoucida*	0.662	33
A. salmonicida **	*A. s.* ss *masoucida*	0.532	42
A. sobria	no identification	-	41
A. sobria *	no identification	0.201	45
A. sobria **	no identification	-	61

[1] absence of an asterik indicates strains were suspended in 0.85% NaCl, strains suspended in marine cation solution (MCS) are marked *, strains suspended in MCS plus yeast extract are indicated by **.

Table 19. Name assigned by Biolog, Biolog similarity level, and number of positive wells in the MicroPlate for *Photobacterium* strains suspended in different salt solutions.

Strain[1]	Name assigned by Biolog (similarity >0.500)	Similarity	wells positive
P. angustum	no identification	-	37
P. angustum *	*P. angustum*	0.839	42
P. angustum **	*P. angustum*	0.633	39
P. leiognathi	no identification	-	32
P. leiognathi *	*P. leiognathi*	0.653	43
P. leiognathi **	*P. leiognathi*	0.848	40
P. phosphoreum	*V. ordalii*	0.606	21
P. phosphoreum *	no identification	-	30
P. phosphoreum **	no identification	-	31

[1] absence of an asterick indicates strains were suspended in 0.85% NaCl, strains suspended in marine cation solution (MCS) are marked *, strains suspended in MCS plus yeast extract are indicated by **.

Table 20. Biolog similarity level, and number of positive wells in the MicroPlate for *Vibrio* and *Listonella* strains identified correctly (similarity >0.500) in all suspending salts solutions.

Strain[1]	Similarity	wells positive
V. alginolyticus	0.905	56
V. alginolyticus *	0.927	55
V. alginolyticus **	0.660	52
L. anguillarum	0.616	48
L. anguillarum *	0.626	49
L. anguillarum **	0.785	48
V. carchariae	0.916	52
V. carchariae *	0.811	55
V. carchariae **	0.806	57
V. cincinnatiensis	0.576	39
V. cincinnatiensis *	0.716	42
V. cincinnatiensis **	0.780	52
L. damsela	0.715	36
L. damsela *	0.820	37
L. damsela **	0.749	40
V. fluvialis	0.970	68
V. fluvialis *	0.839	72
V. fluvialis **	0.520	58

Note: Table 20 is continued on the next page.

Table 20. Continued.

V. furnissii	0.945	69
V. furnissii *	0.812	72
V. furnissii **	0.798	63
V. mediterranei	0.799	57
V. mediterranei *	0.789	64
V. mediterranei **	0.734	67
V. natriegens	0.741	62
V. natriegens *	0.657	65
V. natriegens **	0.579	66
L. pelagia	0.792	47
L. pelagia *	0.807	51
L. pelagia **	0.716	54
V. proteolyticus	0.875	56
V. proteolyticus (r)	0.904	58
V. proteolyticus *	0.904	60
V. proteolyticus **	0.793	53

[1] absence of an asterik indicates strains were suspended in 0.85% NaCl, strains suspended in marine cation solution (MCS) are marked *, strains suspended in MCS plus yeast extract are indicated by **, (r) marks a replicate of the strain suspended in 0.85% NaCl.

Table 21. Name assigned by Biolog, Biolog similarity level, and number of positive wells in the MicroPlate, for *Vibrio* strains that gave an accurate identification (similarity >0.500) in the standard suspension salt (0.85% NaCl) and in one of the marine cation solutions (MCS or MCSpY).

Strain[1]	Name assigned by Biolog (similarity >0.500)	Similarity	Wells positive
V. aestuarianus	*V. aestuarianus*	0.900	50
V. aestuarianus *	*V. aestuarianus*	0.520	53
V. aestuarianus **	no identification	0.386	52
V. diazotrophicus	no identification	0.368	47
V. diazotrophicus (r)	*V. diazotrophicus*	0.671	53
V. diazotrophicus *	no identification	0.277	45
V. diazotrophicus **	*V. diazotrophicus*	0.559	39
V. mimicus	*V. mimicus*	0.720	21
V. mimicus *	*V. mimicus*	0.782	24
V. mimicus **	no identification	0.437	39
V. vulnificus	*V. vulnificus*	0.706	48
V. vulnificus *	*V. vulnificus*	0.772	45
V. vulnificus **	no identification	0.497	30

[1] absence of an asterick indicates strains were suspended in 0.85% NaCl, strains suspended in marine cation solution (MCS) are marked *, strains suspended in MCS plus yeast extract are indicated by **,(r) marks a replicate of the strain suspended in 0.85% NaCl.

Table 22. Name assigned by Biolog, Biolog similarity level, and number of positive wells for *Vibrio* strains that were correctly identified (similarity >0.500) only when suspended in the standard 0.85% NaCl solution.

Strain[1]	Name assigned by Biolog (similarity >0.500)	Similarity	Wells positive
V. campbellii	*V. campbellii*	0.657	38
V. campbellii *	no identification	0.334	47
V. campbellii **	*V. splendidus* 2	0.563	40
V. harveyi	*V. harveyi* A	0.856	48
V. harveyi *	no identification	0.484	60
V. harveyi **	no identificaton	0.326	63
V. hollisae	*V. hollisae*	0.525	21
V. hollisae *	no identification	0.494	36
V. hollisae **	no identification	-	46
V. splendidus	*V. splendidus* 2	0.62	32
V. splendidus *	no identification	0.353	42
V. splendidus **	no identification	-	43
V. tubiashii	*V. tubiashii*	0.799	34
V. tubiashii *	no identification	-	50
V. tubiashii **	no identification	-	58

[1] absence of an asterick indicates strains were suspended in 0.85% NaCl, strains suspended in marine cation supplement solution (MCS) are marked *, strains suspended in MCS plus yeast extract are indicated by **.

Table 23. Name assigned by Biolog, Biolog similarity level, and number of wells positive in the MicroPlate for *Vibrio* strains not included in the Biolog data base.

Strain[1]	Name assigned by Biolog (similarity >0.500)	Similarity	wells positive
V. costicola	no identification	-	13
V. costicola *	*Pas. bettyae*	0.549	22
V. costicola **	*V. cholerae*	0.622	32
V. fisheri	*Aer.* med-1k DNA 5A	0.63	3
V. fisheri *	no identification	-	4
V. fisheri **	no identification	-	49
V. gazogenes	no identification	-	55
V. gazogenes *	no identification	-	58
V. gazogenes **	no identification	-	58
V. logei	no identification	-	27
V. logei *	no identification	-	36
V. logei **	no identification	-	21
V. marinus	no identification	-	20
V. marinus *	*Aer.* DNA 11	0.502	24
V. marinus **	no identification	-	34
V. nereis	*V. ordalii*	0.634	15

Note: Table 23 is continued on the next page.

Table 23. Continued.

V. nereis *	no identification	-	42
V. nereis **	*V. alginolyticus*	0.548	48
V. nigrapulchritudo	*P. phosphoreum*	0.54	13
V. nigrapulchritudo *	no identification	-	35
V. nigrapulchritudo **	no identification	-	35
V. orientalis	no identification	-	4
V. orientalis *	*V. tubiashii*	0.808	31
V. orientalis **	*V. pelagius* I	0.628	42
V. salmonicida	no identification	-	18
V. salmonicida *	no identification	-	22
V. salmonicida **	no identification	-	28

[1] absence of an asterick indicates strains were suspended in 0.85% NaCl, strains suspended in marine cation supplement (MCS) solution are marked *, strains suspended in MCS plus yeast extract are indicated by **.

Table 24. Name assigned by Biolog, Biolog similarity level, and number of positive wells in the MicroPlate for remaining *Vibrio* strains.

Strain[1]	Name assigned by Biolog (similarity >0.500%)	Similarity	wells positive
V. metschnikovii	*Aer.* DNA 11	0.626	19
V. metschnikovii (r)	*Aer.* DNA 11	0.532	24
V. metschnikovii *	no identification	-	12
V. metschnikovii **	*Aer.* DNA 11	0.592	10
V. ordalii	no identification	0.45	20
V. ordalii *	*V. splendidus* 2	0.617	25
V. ordalii **	*V. splendidus* 2	0.908	29
V. parahaemolyticus	no identification	-	32
V. parahaemolyticus *	*V. parahaemolyticus*	0.731	46
V. parahaemolyticus **	*V. parahaemolyticus*	0.785	47

[1] absence of an asterick indicates strains were suspended in 0.85% NaCl, (r) markes a replicate of the strain suspended in 0.85% NaCl, strains suspended in marine cation supplement solution (MCS) are marked *, strains suspended in MCS solution plus yeast extract are indicated by **.

Two of three *Photobacterium* strains were identified by Biolog but only when they were treated with the sea salts solution (MCS) (Table 19). The response of all three species were best in the MCS solution as indicated by the largest number of positive wells for this suspending solution. All of the *Photobacterium* strains suspended in the different solutions gave "breathprints" that resulted in them clustering together in Figure 1 (cluster a) with the exception of *P. phosphoreum*, suspended in saline, which was found in cluster c. In all cases the cells suspended in MCS solutions resulted in "breathprints" that gave tight clusters. It was concluded that the MCS suspending salts was best for the strains of *Photobacterium* although *P. phosphoreum* was not identified.

Table 20 lists the Biolog similarity level and number of positive wells in the microplate for the 10 *Listonella* or *Vibrio* reference strains that were identified correctly using all suspending salt solutions. It can be seen in Figure 1 that the three breathprint patterns, as a result of the three treatments for each of these strains, were tightly clustered together. *V. alginolyticus*, suspended in MCSpY, is an exception to this as it clustered a little less tightly than the others. Using *V. proteolyticus* as a control it was demonstrated that with each

independent trial run of a strain, using the Biolog system, the number of positive wells can vary, and thus the Biolog similarity level, at which the strain is identified, can change. This species varied from 56 to 58 positive wells with the Biolog similarity level increasing from 0.875 to 0.904 for separate Biolog identifications. Although these bacteria in any of the suspending salts had acceptable identification levels, four species had the highest Biolog identification similarities when they were suspended in saline, three in MCS, and two in MCSpY. The number of positive wells does not correspond with this because one species had the most positive wells in saline, three had the most in MCS, and six had the most in MCSpY.

Table 21 lists the Biolog test results for four *Vibrio* strains that were accurately identified, but generally only at an acceptable Biolog similarity level when suspended in saline and one of the marine cation solutions (MCS or MCSpY). Figure 1 shows how three of these strains (*V. aestuarianus*, *V. mimicus*, and *V. diazotrophicus*) had breathprints that resulted in tight clustering for strains in the three salts solutions. *V. vulnificus* (Table 21) is an exception to this. When it was suspended in MCSpY it did not cluster with the same strains suspended in salt solutions. This may be indicative of the fact

that this suspending salt resulted in significantly fewer positive wells than the other two suspending salts (Table 21). When *V. diazotrophicus* was suspended a second time in 0.85% NaCl solution the similarity level increased from an unacceptable level (0.386) to a reliable identification (0.671) thus showing the variation between independent trials when using the Biolog system.

There were five *Vibrio* strains correctly identified by Biolog (Biolog sim. >0.500) only when suspended in saline (Table 22). An interesting relationship exists between these strains. Strains that had been suspended in the MCS and MCSpY salts clustered together while the strains suspended in saline were on their own within the larger cluster, or in another cluster (Fig. 1). Most of these strains had the most positive wells when they were suspended in MCSpY (Table 22). An exception to the above was *V. campbellii* which had the greatest number of positive wells when suspended in MCS. *V. campbellii* also had a different clustering pattern from the other strains in that the 0.85% NaCl and MCS suspending salts resulted in tighter clusters than MCS and MCSpY. It is interesting to note here that the *V. campbellii* breathprints clustered tightly with those of *V. splendidus* because their carbon source utilization patterns were

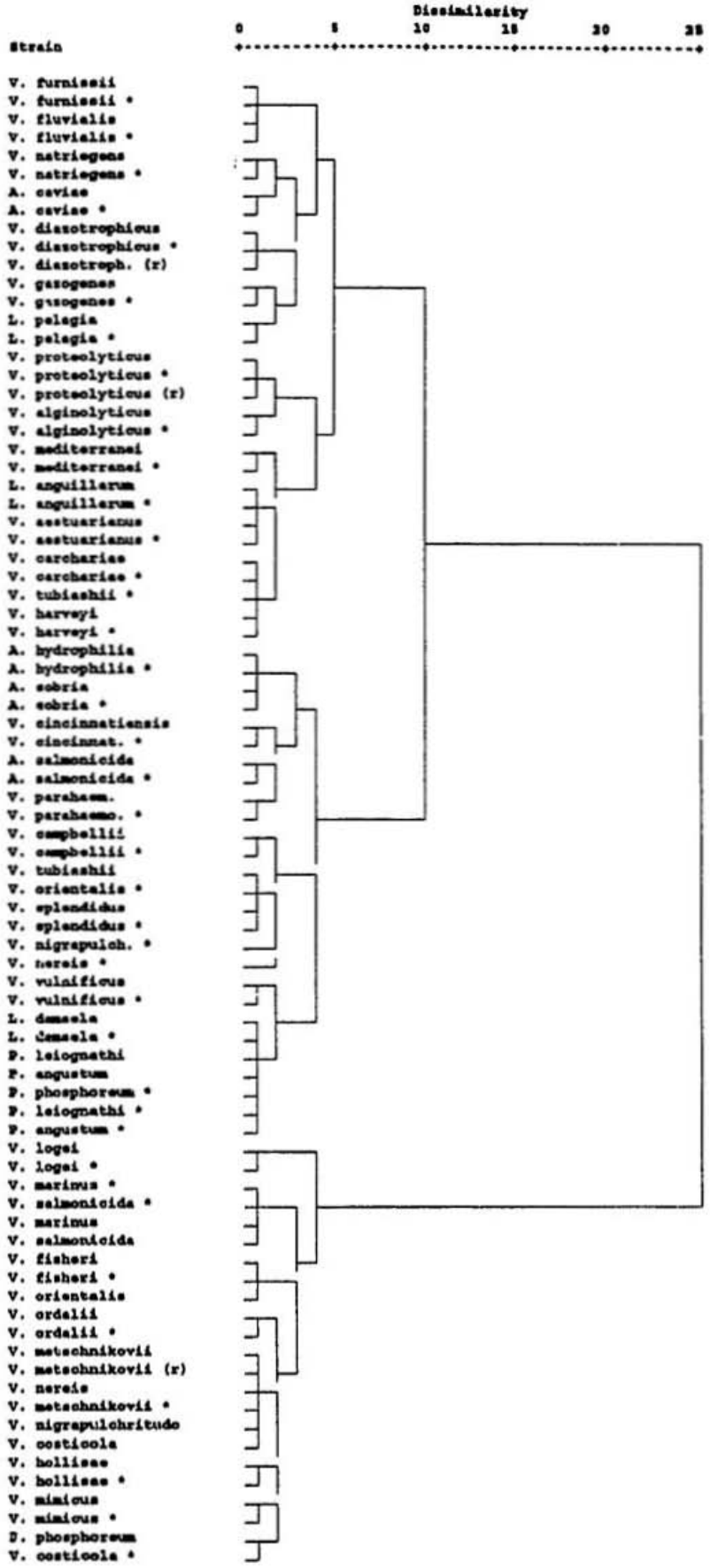
very similar. This was strengthened as *V. campbellii* was identified as *V. splendidus* 2 when it was suspended in MCSpY.

Table 23 lists the Biolog results for the nine species not included in the Biolog data base. Because these strains are not included in the data base they cannot be identified using the Biolog system. The breathprints of these strains were observed to be more extensive when marine cation solutions were used to suspend the bacteria. Six strains had the most positive wells in MCSpY, two strains had an equal number of positive wells in both the MCSpY and the MCS, while one strain responded the best in MCS alone. According to the treatment, these strains were distributed throughout the dendrogram (Figure 1). Three of the species clustered together reguardless of the treatment. Three more species treated with MCS and the MCSpY suspending salts clustered together while strains subjected to the saline treatment were relocated in other clusters. *V. fisheri* had a marked increase in the number of positive wells when suspended in MCSpY as indicated by the separate location of the organism, on the dedrogram, when suspended in this solution. *V. marinus* was located within a cluster with *V. salmonicida* (cluster c), and *V. orientalis* was found in three separate clusters [(saline) cluster d, (MCS) cluster b, and (MCSpY) cluster h].

Of the three remaining species, *V. parahaemolyticus* (Table 24) was correctly identified only when suspension salts including MCS were used. This is further illustrated when these two clustered together (cluster a) while the culture from the saline suspension clustered in c (Figure 1). The most positive wells for this strain occurred in MCSpY. *V. metschnikovii* was not identified correctly by Biolog under any circumstances. It was consistently, although variably, low in the number of positive wells. This is reinforced by the tight clustering of strains subjected to any of the treatments (Figure 1). *V. ordalii* suspensions, in any of the salt solutions also resulted in tight clustering (Figure 1).

Figure 2 is a dendrogram of the results obtained for cultures suspended only in the saline and MCS suspending salts. It can be seen that 28 of 39 strains, suspended in these solutions, clustered very tightly together indicating that the treatment of strains with the sea salts ultimately did not change the results a great deal from the perspective of identification and classification.

Figure 2. Dendrogram showing numerical analysis of *Vibrionaceae* reference cultures suspended in 0.85% NaCl, and MCS (*). Note: the (r) indicates a replicate of the reference culture suspended in 0.85% NaCl.


Dissimilarity
0
5
10
15
20
25
Strain
V. furnissii
V. furnissii *
V. fluvialis
V. fluvialis *
V. natriegens
V. natriegens *
A. caviae
A. caviae *
V. diazotrophicus
V. diazotrophicus *
V. diazotroph. (r)
V. gazogenes
V. gazogenes *
L. pelagia
L. pelagia *
V. proteolyticus
V. proteolyticus *
V. proteolyticus (r)
V. alginolyticus
V. alginolyticus *
V. mediterranei
V. mediterranei *
L. anguillarum
L. anguillarum *
V. aestuarianus
V. aestuarianus *
V. carchariae
V. carchariae *
V. tubiashii *
V. harveyi
V. harveyi *
A. hydrophilia
A. hydrophilia *
A. sobria
A. sobria *
V. cincinnatiensis
V. cincinnat. *
A. salmonicida
A. salmonicida *
V. parahaem.
V. parahaemo. *
V. campbellii
V. campbellii *
V. tubiashii
V. orientalis *
V. splendidus
V. splendidus *
V. nigrapulch. *
V. nereis *
V. vulnificus
V. vulnificus *
L. damsela
L. damsela *
P. leiognathi
P. angustum
P. phosphoreum *
P. leiognathi *
P. angustum *
V. logei
V. logei *
V. marinus *
V. salmonicida *
V. marinus
V. salmonicida
V. fisheri
V. fisheri *
V. orientalis
V. ordalii
V. ordalii *
V. metschnikovii
V. metschnikovii (r)
V. nereis
V. metschnikovii *
V. nigrapulchritudo
V. costicola
V. hollisae
V. hollisae *
V. mimicus
V. mimicus *
P. phosphoreum
V. costicola *

III. Identification of regional *Vibrionaceae* strains using the Biolog system:

Table 25 lists the name assigned by Biolog, the similarity level, and the number of positive wells in the MicroPlate for the 80 regional strains. Results for the regional strains were combined with the results from the reference strains (both were suspended in salts containing MCS) to produce a dendrogram (Figure 3). Table 26 lists the source of the strains, number, name of the regional strain if one was assigned by Biolog, and the cluster membership of the strains. Figure 4 gives the same information as Figure 3 but includes the names of those regional strains identified by Biolog. An identification was obtained for 36% of the regional strains by using the Biolog system.

Table 26 is a synopsis of the information in Figure 4 of which the major points of interest (ie. cluster content) are outlined below:

Cluster A contains mainly type or reference cultures and strains from alga. Three of these regional strains were identified by Biolog as *V. vulnificus*, two as *V. harveyi*, and one as *V. tubiashii*.

Cluster B can be further subdivided into subclusters, B1 and B2. B1 contains mostly reference cultures. Subcluster B2

Table 25. Name assigned by Biolog, Biolog similarity level, and number of positive wells in the MicroPlate for the 80 regional strains.

Strain no.	Name assigned by Biolog (similarity >0.500)	Similarity	wells positive
1	no identification	-	32
2	no identification	-	34
3	no identification	-	27
4	no identification	-	43
5	*Psychrobacter immobilis*	0.600	26
6	no identification	-	38
7	no identification	-	39
8	no identification	-	34
9	Pseudomonas alcaligenes A	0.566	30
10	no identification	-	30
11	*Psychrobacter immobilis*	0.780	20
12	no identification	-	31
13	no identification	-	35
14	no identification	-	39
15	no identification	-	36
16	no identification	-	52
17	no identification	-	35
18	no identification	-	32

Note: Table 25 is continued on the next page.

Table 25. Continued.

19	no identification	-	28
20	no identification	-	32
21	no identification	-	49
22	*Vibrio vulnificus*	0.512	36
23	*Pseudomonas stutzeri*	0.528	30
24	*Vibrio vulnificus*	0.591	37
25	*Psychrobacter immobilis*	0.525	29
26	no identification	-	35
27	no identification	-	36
28	*Vibrio tubiashii*	0.606	39
29	no identification	-	48
30	no identification	-	55
31	*Vibrio harveyi* B	0.616	30
32	*Pseudomonas stutzeri*	0.634	33
33	*Psychrobacter immobilis*	0.547	28
34	no identification	-	6
35	*Vibrio vulnificus*	0.591	36
36	no identification	-	36
37	no identification	-	31
38	*Pseudomonas stutzeri*	0.548	31

Note: Table 25 is continued on the next page.

Table 25. Continued.

39	*Shewanella putrefaciens* A	0.804	15
40	*Psychrobacter immobilis*	0.785	26
41	*Psychrobacter immobilis*	0.717	29
42	no identification	-	41
43	no identification	-	30
44	*Pseudomonas stutzeri*	0.565	34
45	*Shewanella putrefaciens* D	0.667	29
46	*Shewanella putrefaciens* D	0.704	41
47	no identification	-	38
48	no identification	-	40
49	no identification	-	38
50	no identification	-	48
51	no identification	-	37
52	*Psychrobacter immobilis*	0.511	28
53	no identification	-	27
54	*Vibrio tubiashii*	0.570	46
55	*Psychrobacter immobilis*	0.539	30
56	no identification	-	3
57	no identification	-	45
58	*Vibrio harveyi* B	0.641	40

Note: Table 25 is continued on the next page.

Table 25. Continued.

59	no identification	-	55
60	no identification	-	31
61	*Deleya marina*	0.614	43
62	no identification	-	57
63	no identification	-	49
64	no identification	-	56
65	no identification	-	58
66	no identification	-	23
67	Shewanella putrefaciens D	0.742	28
68	*Shewanella putrefaciens* D	0.755	24
69	no identification	-	27
70	*Shewanella putrefaciens* A	0.596	24
71	*Shewanella putrefaciens* A	0.662	25
72	*Psychrobacter immobilis*	0.831	21
73	no identification	-	30
74	no identification	-	38
75	no identification	-	35
76	no identification	-	33
77	no identification	-	40
78	no identification	-	47
79	no identification	-	41
80	*Shewanella putrefaciens* D	0.753	33

Figure 3. Dendrogram showing numerical analysis of *Vibrionaceae* reference cultures and the 80 regional strains (all strains suspended in MCS).

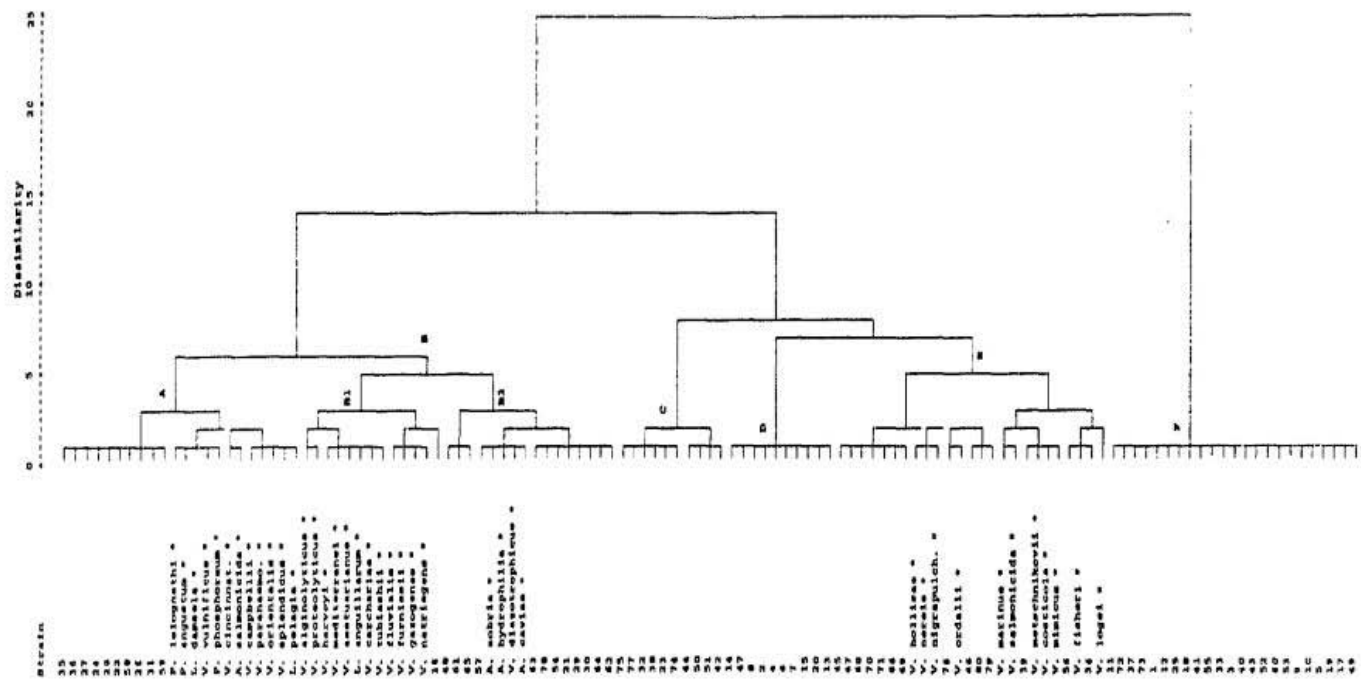

Dissimilarity
0
5
10
15
20
25
A
B
B1
B2
C
D
E
F
strain
lelognathi
ingratus
delafieldi
validilucus
phosphoreum
cincinnat.
salmonicida
carnosus
perfringens
orientalis
splendidus
pelagia
proteolyticus
harveyi
mediterranei
anguillarum
carchariae
tubiashii
fluvialis
gazogenes
marinogena
sobria
hydrophila
alvei
hollisae
sacsa
sp.
ordalii
marinus
salmonicida
weihenstephanensis
mesentericus
simiae
fisheri
logei

Table 26. Source of the strains, number of strains from the source, name assigned by Biolog and original isolate number for regional strains identified in the defined clusters of Figure 4.

Cluster		Source of the Strain	No.[1]	Strains named by Biolog	
				Name assigned	Number[2]
A		reference	12	not applicable	-
		alga	10	*V. vulnificus*	22,24,35
				V. harveyi B	31,58
				V. tubiashii	28
B	B1	reference	12	not applicable	-
		alga	1	not applicable	-
	B2	reference	4	not applicable	-
		alga	11	*D. marina*	61
				V. tubiashii	54
		scallops	1	not applicable	-
C		alga	5	*P. stutzeri*	23,32
		scallops	6	*P. stutzeri*	38,44
D		alga	10	not applicable	-
E		reference	11	not applicable	-
		alga	2	not applicable	-
		scallops	12	*S. putrefaciens* D	45,46,67 68,80
				S. putrefaciens A	39,70,71

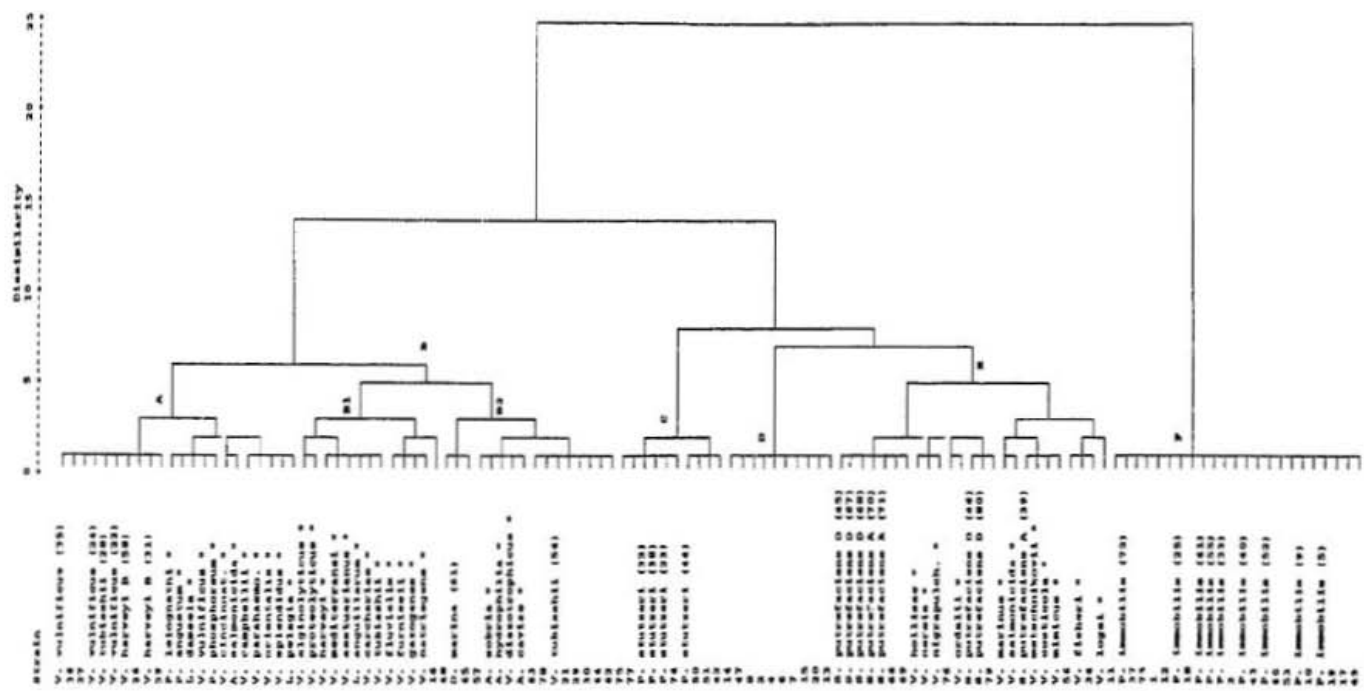
Note: Table 26 is continued on the next page.

Table 26. Continued.

F	alga	18	*P. immobilis*	5,11,25, 33,55
			P. alcaligenes A	9
	scallops	5	*P. immobilis*	40,41,72

[1] No. of strains under consideration.
[2] Number assigned to the strain.

Figure 4. Dendrogram showing numerical analysis of *Vibrionaceae* reference cultures and regional strains (all strains suspended in MCS). Names assigned to the regional strains by the Biolog system are included, the original numbers assigned to these strains are given in parentheses.


Dissimilarity
A
B
B1
B2
C
D
E
F

contains four reference cultures three of which are of the genus *Aeromonas*, the remainder of this cluster, except one from scallop, are strains isolated from alga. Two of these isolates from alga were identified by Biolog. One was identified as *D. marina* and the other as *V. tubiashii*. Cluster C contains regional strains from two sources. Six were isolated from scallops and four from *Alaria*. Two strains from each of these groups were identified as *P. stutzeri*.

Cluster D is composed entirely of regional strains from *Alaria*, none of which was identified by Biolog.

Cluster E contains eleven type or reference cultures, 12 strains from scallop, and two strains from *Alaria*. The strains from *Alaria* were not identified by Biolog, but eight of 12 strains from scallop were. Three of these strains from scallop were identified as *Shewanella putrefaciens* A and five were named *Shewanella putrefaciens* D.

Cluster F consisted mainly of strains from *Alaria* with five strains from scallop intermixed. Three of the strains from scallop were named *P. immobilis* by Biolog as were six strains from kelp. One of the strains from *Alaria* was assigned the name *P. alcaligenes* A.

IV. A Comparison of classifications resulting from Biolog and Bergey's *Manual* criteria:

A dendrogram (Figure 5) was produced of the regional and reference strains using data collected by Martin-Kearley (1992) using classical methods. For the study presented here the data consisted of a series of test results fashioned from Table 5.52a of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994). The various clusters of the dendrogram in Figure 5 have been assigned numbers for clarity when this dendrogram is to be compared with those produced using Biolog data that were given letters. The content of the clusters of Figure 5 is summarized in Table 27. From both the table and the figure it is apparent that the content of the specified clusters is relatively homogeneous, meaning that the defined clusters tend to contain mostly strains from one particular source. Clusters 1 and 4 (Figure 5) are good examples of this uniformity because cluster 1 contains only strains from alga while cluster 4 contains only reference strains. Also, if cluster 5 is divided into two subclusters, 5A and 5B, a similar phenomenon occurs. Cluster 5A contains strains from alga and cluster 5B contains reference cultures. Clusters 3 and 6 are also relatively uniform. Cluster 3 contains one strain from alga and the remaining reference

Figure 5. Dendrogram showing numerical analysis of *Vibrionaceae* reference cultures and regional strains. Data were collected by Martin-Kearley and Gow (1994) and analyzed based on the criteria of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994).

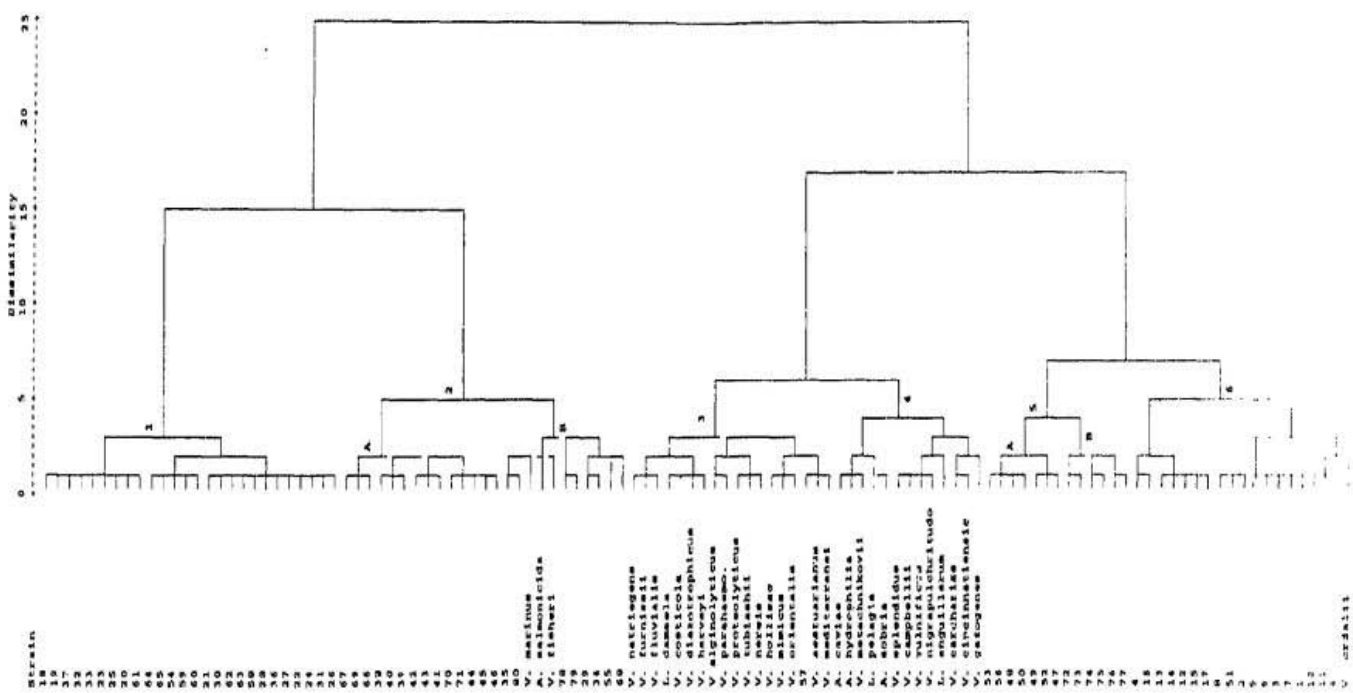

Dissimilarity
0
5
10
15
20
25
Strain
maximum
salmonicida
fisheri
natriegens
furnissii
fluvialis
damsela
costicola
diazotrophicus
alginolyticus
parahaemo.
proteolyticus
tubiashii
orbalis
nereis
mimicus
ordentalis
sp7
aestuarianus
mediterranei
caviae
hydrophilia
metschnikovii
proteolytica
splendidus
campbellii
vulnificus
nigripulchritudo
anguillarum
ordalii
cincinnatiensis
gazogenes
ordalii

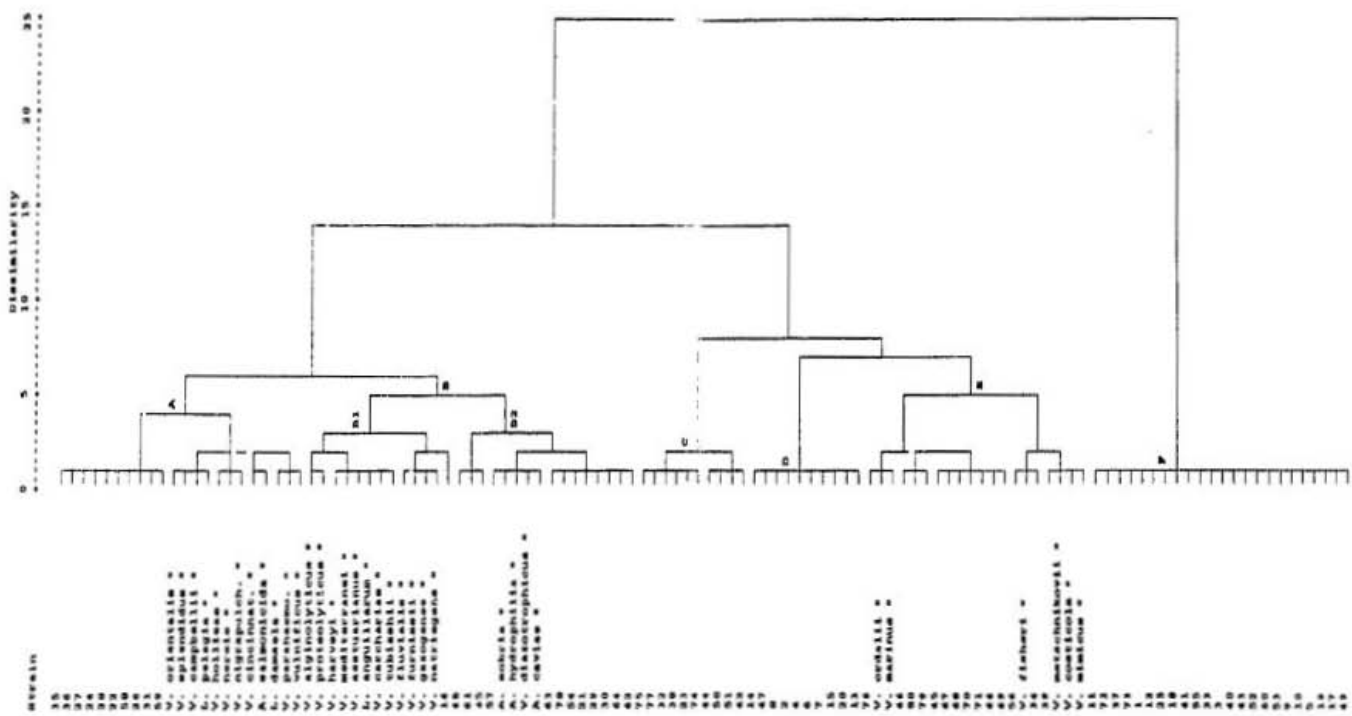
Table 27. Source of the strains, and the number of strains from the source, for the defined clusters of Figure 5 based on the classification of Holt et al. (1994) in *Bergey's Manual of Systematic Bacteriology*.

Cluster in Figure 5		Source	Number of strains
1		alga	26
2	2A	scallop	14
	2B	reference	12
		alga	4
		scallop	4
3		reference	17
		alga	1
4		reference	13
5	5A	alga	6
	5B	scallop	6
6		reference	1
		alga	19

cultures. Cluster 6 has one reference culture (*V. ordalii*) and the remaining are strains from alga. Cluster 2 is more heterogeneous than the clusters described thus far as it contains three reference cultures (*V. marinus, V. fisheri,* and *A. salmonicida*), six strains from alga, and sixteen strains from scallop. If cluster 2 is subdivided into clusters 2A and 2B, the content of these clusters is more homogeneous with 2A containing two strains from alga and twelve strains from scallop and 2B containing the three reference cultures, four strains from alga and four strains from scallop.

It was felt that, before the dendrograms based on the *Bergey's* classification and by the Biolog system could be compared, the strains included in each analysis should be the same. To do this five reference cultures included in the Biolog results of Figure 3 had to be excluded. These were; *V. logei, V. salmonicida* and all *Photobacterium* strains. The exclusion of these five strains resulted in some changes and the resulting dendrogram is shown in Figure 6. As it turned out two of these reference cultures (*V. logei* and *V. salmonicida*) belonged to cluster E of Figure 3 and if one refers to Figure 6 the absence of these strains is apparent. Three of the *Photobacterium* strains were part of cluster A, in Figure 3. The exclusion of

Figure 6. Dendrogram showing numerical analysis of *Vibrionaceae* reference cultures and regional strains (all strains suspended in MCS), based on data collected by the Biolog system. Reference strains included are those also included in the study of Martin-Kearley and Gow (1994).


dissimilarity
0
5
10
15
20
25
A
B
B1
B2
C
D
E
F
strain
35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74

these strains resulted in some reshuffling of the reference cultures in Figure 6. That is, *V. hollisae*, *V. nereis* and *V. nigrapulchritudo* of cluster E (Figure 3) moved to cluster A of Figure 6. There were also some other rearrangements of strains within the clusters, details of which will not be discussed here.

In order to compare the dendrograms produced using Biolog data (Figure 6) to those produced using classical data (Figure 5) a third dendrogram was produced (Figure 7). Figure 7 is a dendrogram produced using *Bergey's* classification with a label alongside every strain indicating to which cluster each strain belonged, in Figure 6 which is based on Biolog results. An examination of this label column of Figure 7 indicates that there appears to be a mixture of Biolog cluster membership within the clusters defined by the *Bergey's* classification. The mixing of the strains within the clusters is further illustrated by Table 28. It can be seen from Table 28 that the clusters numbered one through six of Figure 5 (based on *Bergey's* results), contain strains from a mixture of clusters based on Biolog data (Figure 6). For example, clusters 4 and 5 of Figure 7 both contain twelve strains. In cluster 5 the strains were found to be widely dispersed in the dendrogram produced based on Biolog results (Figure 6). As indicated by Table 28, one of these strains was

Figure 7. Dendrogram showing numerical analysis of *Vibrionaceae* reference cultures and regional strains. Data were collected by Martin-Kearley and Gow (1994) based on the criteria of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994). Cluster membership of the strains based on Biolog system data (Figure 6) are given. Regional strains are identified by their originally assigned numbers, reference cultures are indicated by ref.

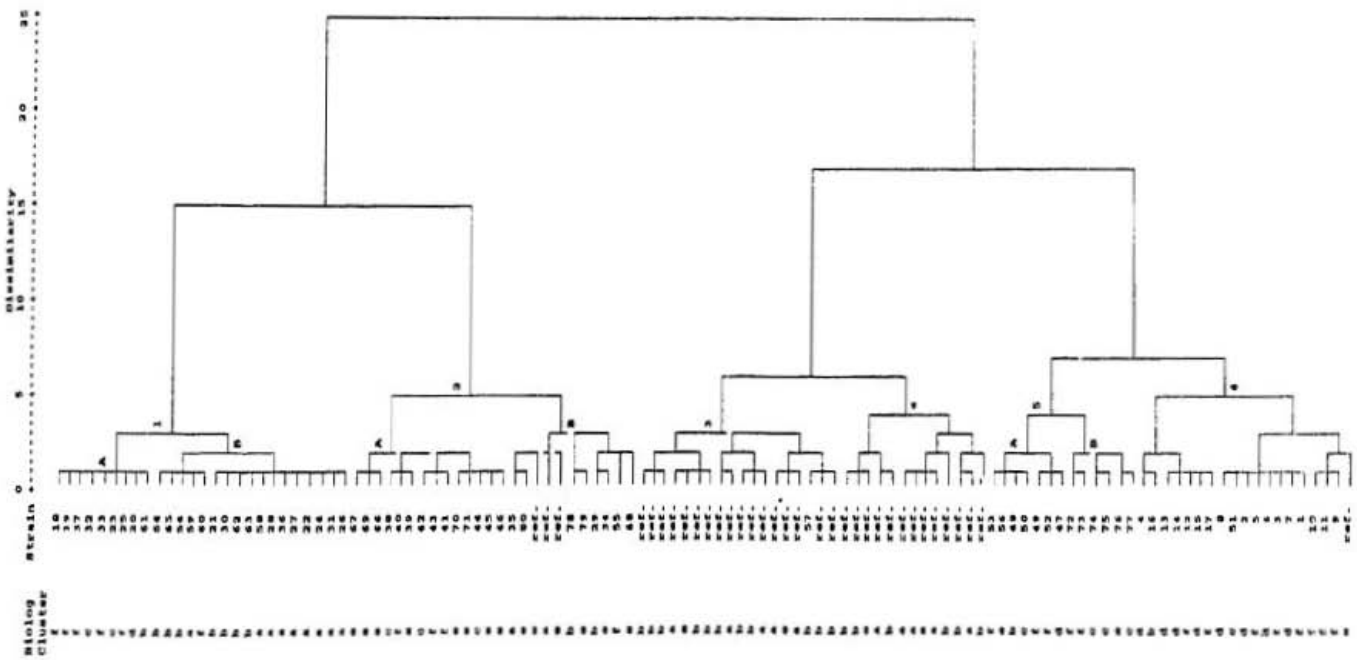

Dissimilarity
Strain
Biolog Cluster

Table 28. Cluster membership (and number) of strains clustered based on Biolog data (Figure 6) within the defined clusters based on data collected by Martin-Kearley (1992), for tests described by Holt et al. (1994) in *Bergey's Manual of Systematic Bacteriology* (Fig.5).

Cluster of Figure 5 (*Bergey's* results)	Cluster of Figure 6 (Biolog results)	Number of strains from specified Biolog cluster
1	A	9
	B	8
	C	2
	D	1
	F	6
2	A	2
	B	2
	C	3
	E	14
	F	4
3	A	5
	B	11
	E	2
4	A	6
	B	6

Note: Table 28 is continued on the next page.

Table 28. Continued.

5	B	1
	C	4
	D	1
	E	2
	F	4
6	B	1
	C	1
	D	8
	E	1
	F	9

from each of clusters B and D, two were from cluster E, and four of these strains were each from clusters C and F. The twelve strains from cluster 4, on the otherhand, contained six strains from each of clusters A and B of Figure 6.

To determine if there was a significant difference in the clusters generated from the Biolog data, and those generated based on the *Bergey's*, a Pearson Chi-Square Test was performed. Table 29 lists the number of strains observed to be similar in the clusters generated from Biolog data and those generated from Bergey's data.

The Pearson Chi-Square p-value is < 0.01 (χ^2=119.91, DF=25). This indicates that there is an association between the two dendrograms; they are significantly dependent on one another. That is, the result of one is dependent on the other or the results of one dengrogram can predict that of the other.

Table 29. Number of strains observed in the clusters generated from the Biolog data (Figure 6) that occur in the same clusters (Figure 5) generated from data based on the criteria of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994).

Biolog Clusters	Clusters Generated by *Bergey's* (1994) Classification					
	1	2	3	4	5	6
A	9	2	5	6	0	0
B	8	2	11	6	1	1
C	2	3	0	0	4	1
D	1	0	0	0	1	8
E	0	14	2	1	2	0
F	6	4	0	0	4	6

DISCUSSION

The family *Vibrionaceae* is one of the most important bacterial groups in marine environments. Members of this family often predominate in the bacterial flora of seawater, plankton, and fish. In a survey carried out in the West Pacific Ocean, vibrios accounted for nearly 80% of the bacterial population in surface seawater (Kita-Tsukamoto et al. 1993). The members of this group have been the subject of many taxonomic studies and this group is among the best-established marine bacterial taxons.

The number of described species in the family *Vibrionaceae*, particularity the genus *Vibrio*, has been expanding rapidly. It has increased from five in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974) to in excess of 34 (of which some are of uncertain taxonomic standing), in the most recent edition of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994). As the number of species increased in this genus so did the number of determinative tests required to differentiate and identify the members. For example, the eighth edition of *Bergey's Manual* lists 31 determinative tests to differentiate between the 5 listed *Vibrio* species, while the ninth edition of *Bergey's* (1994) lists 81 determinative tests to differentiate the 34 species. Therefore, identifying a member

of this group by "classical methods" is a very labour intensive and slow process.

A main objective of this study was to evaluate the Biolog system's capacity to correctly identify reference strains and to characterize regional strains isolated from Newfoundland coastal waters. Another objective was to compare a Biolog produced classification of these strains with one based on "classical methods" using tests recommended in the ninth edition of *Bergey's Manual*.

To undertake this task, reference cultures (ATCC) of the family *Vibrionaceae* and 80 regional strains were used. These regional strains were isolated from kelp and scallop and were identified as *Vibrio* in earlier studies (Hollohan, 1982; Hollohan et al. 1986; and Martin-Kearley and Gow, 1994).

I. Verification of the authenticity of the *Vibrionaceae* reference cultures:

Verification was done to address several concerns. Bacterial strains, maintained in subculture and put through several passages of media, may decrease in vigour or lose some of their original properties (Bryant et al. 1986). Some may become mislabelled or contaminated. Considering that many of the

reference cultures used in this study had been used in previous studies and preserved under sterile mineral oil or by lyophilization their purity and metabolic vigour had to be ensured. The type strains used in this study were analyzed for purity by performing the classical tests recommended for vibrios in the determinative table of Farmer and Hickman-Brenner in Vol.3 of *The Prokaryotes* (Balows et al. 1992). Biolog identification results were used in conjunction with the classical test results to verify that the cultures had properties appropriate to the species to which they were assigned. If a strain had determinative test results in close agreement to those outlined by Farmer and Hickman-Brenner (1992), or was correctly identified by the Biolog system, it was considered to be correctly labelled and pure. Strains which were problematic with respect to one, or both, of these classifications were suspect and replaced with new cultures from the ATCC.

Twenty-three reference cultures, (59%), were correctly identified by using the Biolog system. Five of these correctly identified reference cultures, along with another nine which were not contaminated, were new or replacement cultures from the ATCC. This, in total, accounted for 82% of the *Vibrionaceae* type cultures studied. Of the remaining reference cultures 10% were

Aeromonas strains. *Aeromonas* species are not listed in the determinative table of Farmer and Hickman-Brenner (1992) and were not suspected as being impure. Therefore, these strains were not verified using this preliminary method. The three remaining reference strains were not included in the Biolog system's data base and their purity could not be verified using this system. They did however have determinative test results very similar to those listed in the determinative table of Farmer and Hickman-Brenner (1992). For example, in this study, both *V. gazogenes* and *V. logei* had determinative test results that exactly matched those outlined in the determinative table of Farmer and Hickman-Brenner (1992). *V. ordalii* differed from the expected test results in two tests, which were fermentation of lactose and sucrose, and this was not considered to indicate impurity. As a result, all of the reference cultures were considered to be pure and to be representative of the species.

In a cross-reference between the two classifications a strain may be considered pure by one or both of the determinative criteria. However, in some instances, the classical test results observed differed from the test results reported in *The Prokaryotes* (Balows et al. 1992). This may be indicative of intralaboratory differences in reading the test results or it may

indicate a strain which has lost its metabolic vigour and, as a result its metabolic characteristics (Bryant et al. 1986). Misinterpretation of "classical" test results may lead to incorrect or no identification by researchers using this method only. The record of the "classical" test results presented in this study can serve as a reference for problematic determinative tests of the *Vibrionaceae* in future studies. The Biolog system should not be as sensitive to misinterpretation because strains are identified by a standardized method. *V. hollisae*, a fresh type culture (ATCC), was correctly identified by Biolog but it differed from the classical determinative test results for lysine decarboxylase (2 and 7-day readings), arginine dihydrolase (7-day reading), sucrose, D-mannitol, maltose, trehalose, and cellobiose fermentation. This strain illustrates how difficult it can be to use classical identification methods. However, a strain may not have been included in Biolog's data base or it may have given an incorrect identification by this system. In these cases classical determinative test results that closely correspond to those that can be expected are invaluable. An example would be *V. nereis*. This strain gave classical test results in very close agreement with those expected but it was not included in Biolog's data base and therefore could not be verified using only this

system. A person can be confident that the breathprint obtained and used in this study is for the correct organism. For comparative purposes a record of the classical determinative test results obtained for the reference strains has been included in this study.

II. Changing the established Biolog test procedures to account for the ion requirements of marine bacteria:

Most of the halophilic vibrios (those other than *V. cholerae* and *V. mimicus*) require more Na^+ for growth and expression of various pathways than is available in standard media (Farmer and Hickman-Brenner, 1992). The addition of marine cations (Na^+, K^+, and Mg^{2+}) when preparing the bacterial suspensions used to determine the identity of the *Vibrio* and *Photobacterium* strains, was to fulfill this requirement. The manufacturers at Biolog, Inc. recognize the fact that many marine bacteria prefer a higher level of salt and will subsequently utilize more carbon sources if they are cultured on marine agar or if they are suspended in 2% to 5% NaCl. It has been stated that this requirement of marine bacteria has been directly responsible for the inadequacies of other rapid multistate systems, if it is not taken into account. As stated in West and Colwell, (1984), "The

use of these kits, prepared according to the manufacturers' instructions, to character_ze *Vibrionaceae* members from the marine environment can result in misidentification (Davis and Sizemore, 1981). It appears that such misidentification occurs because marine isolates grow suboptimally in the recommended suspending fluid which does not contain sufficient sodium chloride and other electrolytes. The studies of Sanyal (1981) support this conclusion, since only non-marine isolates showed good growth and correlation with conventional tests in one commercial kit. It is necessary to modify the electrolyte composition of suspending fluids in some commercial kits for use with marine and estuarine species (MacDonell et al. 1982)."

The Na^+ requirement of marine bacteria is a factor that has been overlooked in the past by the manufacturers of commercial identification kits. For this reason it was a point of interest in this study. The effect of changes to the Biolog protocol to accommodate this requirement on the system's capacity to correctly identify the strains was determined. This will be discussed in following sections.

A. Cultivating media

The procedures manual for the Biolog system (Biolog Inc.,

Hayward, Calif.) stresses the importance of choosing an appropriate agar medium on which to grow the strains. The medium of choice must support growth and promote retention of full metabolic activity. This is necessary for the strains tested to give appropriate metabolic patterns to match those in the GN data base. Biolog recommends that the vast majority of Gram-negative bacteria be grown on either Biolog Universal Growth Medium (BUGM) or Tryptic Soy Agar (TSA) with or without the addition of 5% sheepblood. These conditions are optimal for a broad range of bacteria but may not be optimal for most marine bacteria. To determine if the cultivating medium would have an effect on the identification given by the Biolog system three *Vibrio* reference cultures were cultivated on several growth media. V. *metschnikovii* was not identified under any circumstances, and for this organism the cultivation medium would not have made a difference. Both *V. proteolyticus* and *V. diazotrophicus*, grown on the TSA and YEP media, had insufficient metabolic activity for correct identifications to be made. The remaining media, TSA plus glycerol and BUGM combinations, with or without MCS, were possible choices for cultivating media. From the results of this study it was concluded that either TSA plus glycerol and MCS, or BUGM, would be suitable cultivating media. The TSA plus glycerol

with MCS was chosen for this study because its composition was known and it could be produced in the laboratory.

B. Suspending salts

Of the genera tested, only *Aeromonas* does not have a Na^+ requirement. Therefore, when the marine cation supplement solutions were used to suspend these strains their metabolic capacity was not expected to increase and the number of carbon sources utilized in the Biolog microplates, regardless of suspension solution, was expected to be approximately the same. In the dendrogram, the *Aeromonas* strains suspended in either saline or MCS diluent were located within a single cluster. It was concluded that using suspending salts containing MCS would not, on its own, contribute to an incorrect identification of *Aeromonas* strains.

Three of four *Aermonas* species developed the most positive wells in the microplate when they were suspended in MCSpY. This may reflect the additional vitamins and amino acids supplied in the yeast extract. These may have promoted more metabolic activity or may have supported growth. The metabolic change was significant for *A. hydrophilia* and *A. sobria* when they were suspended in MCSpY because it resulted in their relocation to

different clusters in the cluster analysis. The use of suspending salts supplemented with yeast extract is not recommended for *Aeromonas* strains.

In a phylogenetic study based on sequences of 16S rRNA Kita-Tsukamoto *et al.* (1993) recommended that the members of the genus *Aeromonas*, currently within the family *Vibrionaceae*, be separated into their own family. They suggested *Aeromonas* strains be members of a new family *Aeromonadeae* based on this genus being homogeneous and clearly distinct from the other species of the family *Vibrionaceae*. In the study presented here *Aeromonas* strains clustered separately from other species in the family *Vibrionaceae*. This supports the conclusion that these strains are closely related.

In this study only *A. salmonicida* ss *masoucida* was correctly identified by using the Biolog system and the identification was correct when the organism was suspended in any of the three suspending salt solutions. The highest similarity level, at 0.786, for Biolog identifications was found in the standard 0.85% solution. Although *A. hydrophilia* was not identified correctly by using the Biolog system it was identified correctly in two of three trials in an evaluation of the system by Miller and Rhoden (1991). In a preliminary study by Carnahan et al. (1989) it was

suggested that the Biolog GN Microplate panel may be useful for species identification within this genus. They found that each of 60 clinical *Aeromonas* strains tested could be unambiguously placed in their correct taxonomic positions. Species of this genus have been included in the GN data base since the study of Carnahan et al. (1989) and therefore would be expected to be identified correctly by the Biolog system. The *Aeromonas* strains used had not been tested by classical determinative tests in the present study. However, they did cluster closely together in the dendrogram produced using the Biolog system. This would suggest that problems associated with using the wrong strains should not be expected. From the results of the present study it is apparent that the Biolog system is not free of problems associated with the identification of *Aeromonas* species.

Members of the genus *Photobacterium* are known to have a Na^+ requirement, therefore they would be expected to exhibit more metabolic activity, or more positive wells, in the Biolog microplate when suspended in a marine cation salts supplement. The three *Photobacterium* species, when suspended in MCS supplemented diluent, did exhibit the highest number of positive wells. The yeast extract supplement did not seem to promote more metabolic activity or growth for members of this genus. *P.*

angustum and *P. leiognathi* were identified correctly only when suspended in the MCS or MCSpY. Considering that Biolog's data base is based on carbon source utilization patterns of strains suspended in 0.85% NaCl this result was unexpected. *P. phosphorum* was identified correctly when in the MCS suspending salts and incorrectly identified when 0.85% NaCl was used. The fact that these species clustered tightly is consistent with the study of Kita-Tsukamoto et al. (1993) based on 16S rRNA data. They found that these three species had common sequences which were not identical to the base sequences of other genera and therefore are closely related.

Kita-Tsukamoto et al. (1993) found that the genus *Listonella* was a heterogeneous group with the species being distributed in various subgroups of the genus *Vibrio*. This, plus the fact that this genus is already of uncertain taxonomic standing (Holt et al. 1994), is the basis of the genus *Listonella* being included with the genus *Vibrio* for discussion purposes in this study. Both of these genera are known to have a Na^+ requirement.

Three *Listonella* species and eight *Vibrio* species were correctly identified using all three suspending salt solutions. The highest Biolog similarity levels for these species were, to an extent, inversely related to the highest number of positive

wells. That is, four species had the highest similarities when they were suspended in saline, three in MCS, and two in MCSpY. The highest similarity levels, which was for the strains that were suspended in 0.85% NaCl, was expected because this suspending solution is the one that Biolog used to build the data base.

Five *Vibrio* strains were correctly identified at an acceptable similarity level only when suspended in 0.85% NaCl suspending solution. Four of the *Vibrio* species studied were correctly identified when suspended in 0.85% NaCl and in one of the marine cation supplements. Of the remaining *Vibrionaceae* type cultures (ATCC) included in this study, nine were not included in Biolog system GN database. The remaining three *Vibrio* strains studied did not fit into any of the other categories already discussed. The results indicate that, marginally, saline may be the best suspending salt solution for identification purposes, although it may not be the best choice for investigators interested in classification. This conclusion is based on the observation that the marine strains in this study are more metabolically active when suspended in marine cation supplements rather than the standard 0.85% NaCl.

Because use of 0.85% NaCl is recommended as the suspending

salt one would expect that the percentage of strains correctly identified would be the greatest when these strains were suspended in the standard 0.85% NaCl solution. This is because the computer programme for identification is based on breathprints produced in this manner. It could also be predicted that the highest number of positive wells for these species would be found when marine cations are used in the suspension solution especially when a nutritional supplement, such as yeast was present. It was found that Biolog correctly identified 57% of the marine strains when they were suspended in the standard 0.85% NaCl solution, and 49% and 43% were identified correctly in MCS and MCSpY respectively. Only 12% of these marine strains had the most positive wells when suspended in the 0.85% NaCl solution. This almost doubled (27%) when MCS was used as the suspension solution. This number doubled once again, to 61%, when yeast was included. The MCSpY by far supported the most metabolic activity or growth in the microplates. The number of incorrect identifications of strains was also highest, at 17% in the MCSpY, followed by 0.85% NaCl at 14%, and then MCS with 11%. Although there was not a large difference between the suspension salt treatments in this respect, the error rate was the highest in the MCSpY. This is expected because the metabolic activity was

highest in this solution and there should be more mismatches of breathprints in the database. Forty percent of strains were not identified in either of the MCS solutions as opposed to 28.57% unidentified when the standard NaCl solution was used. This probably resulted from unrecognized breathprints created when these strains became more metabolically active in the marine cation supplement solutions.

When the four *Aeromonas* strains were included in the analysis it was expected that the identifications, for these species, would be correct in the saline solution and the marine cations may, or may not, affect the identifications. It was found that only one of these species was correctly identified and this happened for the three different treatments. In conclusion, suspending *Aeromonas* strains in suspending salts containing marine cation supplement did not have an adverse effect on identification.

Theoretically, replicates should yield identical results in the same laboratory, but differences often occur. Factors that impair intralaboratory reproducibility are reading intervals (24h versus actual 22-26h) and variations in incubation temperature that occur because of inaccurate temperature settings or fluctuations in temperature that occur from shelf to shelf within

air connected incubators. Variation in the reproducibility of tests between laboratories is expected to be larger than variation within a laboratory because of factors other than those mentioned above. Variations can be a result of batch or brand differences among culture media, differences in inoculation techniques, or discrepancies between observing and reading results (D'Amato et al. 1991). Overall, commercial identification systems are more reproducible than their conventional counterparts (D'Amato et al. 1981) because of standardization of methods and microplate reader instrumentation that should minimize or eliminate reader bias.

Because of the potential errors that may be introduced when the Biolog protocol is not exactly followed, three vibrio strains were tested using exact Biolog protocol to test the system's reproducibility. *V. proteolyticus* was identified correctly by the Biolog system at high Biolog similarity levels (0.875 and 0.904) both times the strain was tested using the system. The number of positive wells increased by two in the second trial run. *V. metschnikovii* was incorrectly identified as *Aeromonas* DNA group 2 in both replicates which gave Biolog similarities of 0.626 and 0.532. The number of positive wells increased between replicates from nineteen to twenty-four. *V. diazotrophicus* was

not quite as consistent as it was correctly identified (Biolog similarity 0.671) with fifty-three positive wells in one trial run and was not identified at an acceptable similarity level (Biolog similarity 0.368) in the second trial run which had forty-seven positive wells. Other researchers have found variability in the GN plate test results for the same cultures evaluated at different times. Wong et al.(1992) found that repeated assays with one strain of *Brucella suis*, two strains of *B. melitensis*, and two strains of *B. abortis* gave >95% agreement, and in all cases the species identification remained the same. Hartung and Cilerolo (1991) also found that profiles in the Biolog assay were generally, though not entirely, reproducible. They found that the profile varied in at least one carbon source between replicate assays of the same strain performed on different days (95%, 41/43 strains) and in duplicate readings of the same plate taken within one hour (19%, 16/86). With the manufacturers' recommended criterion of an absorbance of at least 40% greater than the control well to distinguish positive from negative results, some cultures varied from positive to negative for the oxidation of a particular substrate because they gave a relatively weak reaction (Fredrickson *et al.* 1991). This variation, which was found primarily for carbon sources that were

only weakly oxidized, was also found to be a problem by Hartung and Cilerolo (1991) and Vernure *et al.* (1993). Fulthorpe and Wyndham (1991) included a control strain in their study and found the duplicate GN microplate fingerprinting indicated a dissimilarity coefficient of three or lower because of the variabilities in the reactions or their interpretation. In their interpretation this represented the practical limit of resolution of strains.

Miller and Rhoden (1991) studied data on the strains classified as "not identified" or "poor identification" to determine how accurate the system would be if the first choice (highest similarity index) was accepted by the user. They found that 46% of the unidentified strains would have been correct at the species level , which would have improved the performance of the system. If the first choice had been accepted as correct in this study (although the Biolog similarity may have been less than 0.500) then the percentage of correct identifications, when the strains were suspended in the 0.85% NaCl would have dropped to 56%. The percentage correct in suspending salts containing MCS would have improved to 59% and to 54% in MCSpY. This evened out the percentage of correct identifications in each of the suspending salt solutions. This indicated that more species were

identified correctly but at an unacceptable similarity level (< 0.500) when MCS or MCSpY were used as the suspending solution.

Other evaluations of the Biolog system found significantly better identification levels than were found in this study. Klinger et al. (1992) used the system to identify 39 ATCC reference taxa and 45 Gram-negative strains from fresh water samples. They found that 76% of the reference strains were identified to the species level within four to twenty-four hours (note: four hour data were used as well as 24h data) and 93% of the strains from water were identified. Miller and Rhoden (1991) used the Biolog system to identify a diverse group of clinically relevant strains of the family *Enterobactereaceae* and Gram-negative non-*Enterobactereaceae*. Of the Biolog reported identifications 57% were correct at the species level at 24 hours. Although it is not known which version of Biolog they used their results for clinical species are similar to those obtained in this study for a mixture of reference and environmental strains.

The basic principle behind the operation of the Biolog system was developed in 1977 in preliminary work by Bochner and Savageau. They developed an indicator plate for diverse types of substrates and microorganisms. Its essential components

consisted of agar, buffer, growth-supporting nutrients (simulated proteose peptone), a test substrate, and a reducible indicator, in this case TTC. They worked with mutant strains to determine the conditions that would allow colonies to grow without the reduction of tetrazolium. They wanted to promote TTC reduction in colonies capable of catabolizing the test substrate. A low concentration of nutrients was the key factor. They explained it as follows, "The concentration of nutrients in the environment was found to be a key factor influencing TTC reduction. Cells growing in the presence of low concentrations of nutrients employ high-affinity, low-capacity systems to transport these nutrients. In this situation, the nutrients are used for macromolecular synthesis and growth, and catabolic and biosynthetic processes are tightly coupled. As the concentration of nutrients is raised, one or more become sufficiently concentrated to enter the cell, via low-affinity, high-capacity transport systems, and be wastefully catabolized as well as assimilated. High rates of catabolism of "excess nutrients" result in increased electron flow, some of which is diverted on to TTC to produce formazan."

When the three suspending salt solutions were used to suspend and subsequently identify members of the *Vibrionaceae* by the Biolog system there were two overall trends:

(1) The percentage of correct identifications decreased as the suspending solutions were more altered from the standard form; 54% in 0.85% NaCl, 46% in MCS, and 41% in MCSpY.

(2) The percentage of positive wells found in the microplates for the strains approximately doubled when the suspending solution included marine salts; 14% in 0.85% NaCl, 24% in MCS, and 62% in MCSpY.

From this it is apparent that the MCSpY as the suspending solution resulted in the lowest number of correct identifications, but it by far increased the number of positive wells in the Biolog microplate. One of the distinct advantages of the Biolog system over conventional methods is that strains do not need to grow in the assay. They only have to be metabolically active to reduce the tetrazolium violet (Mauchline and Keevil, 1991). Therefore, although the addition of yeast extract appears to be very beneficial by producing breathprints with more positive wells it is not known why this is occurring. That is, the yeast extract may encourage the cells to become more metabolically active and to reduce more substrates, or, the addition of yeast extract may supply an ample supply of nutrients to the basal medium and thus support growth. If the latter is true then the breathprint obtained using this suspending solution

may not be a true picture of the metabolic capacity of the strains, but rather a species growth response. A reason for experimenting with the addition of yeast extract to the suspending salts was because Baumann et al. (1984), as well as Holt et al. (1994), reported that some species of *Vibrio* require organic growth factors when tested for growth on carbon compounds as sole sources of carbon and energy. An example would be some strains of *V. costicola*, *V. logei*, *V. metschnikovii*, and *V. orientalis*. In this study it was of interest to determine if the addition of organic growth factors would have a significant effect on the breathprints of any, a few, or most *Vibrio*. It was concluded that the impact was on a few of the strains tested. It may have its uses for exploring the growth requirements of marine bacteria but would be disadvantageous when using the Biolog system for identification.

III. Using the Biolog MicroStation System to identify regional *Vibrionaceae* strains:

When working with unknowns it is better for a strain not to be identified rather than to be incorrectly identified. In this study, approximately 8% more reference *Vibrionaceae* strains were correctly identified by Biolog when the standard 0.85% NaCl

suspending solution was used instead of MCS. More strains were also incorrectly identified when the standard saline solution was used instead of the MCS. This, in conjunction with the fact that *Vibrionaceae* reference strains are more metabolically active (more positive wells) in the MCS suspending solution, led to MCS being the suspension solution of choice for the regional strains.

The Biolog breathprints for the 80 regional strains, suspended in MCS, were used in conjunction with those obtained for the 39 *Vibrionaceae* type cultures, which had been suspended in MCS, to produce a dendrogram. The reference cultures were found to cluster in groups, as did the strains isolated from scallops.

Using the Biolog system, an identity was assigned to 36% of the regional strains studied. The strains isolated from *Alaria* and assigned a name by the Biolog system were as follows; six strains were identified as *Vibrio* species (three as *V. vulnificus*, two as *V. harveyi* B, and one as *V. tubiashii*), eight were identified as *Psychrobacter immobilis* (these clustered together), and one was identified as *Deleya marina*. Two strains from *Alaria* were identified as *Pseudomonas stutzeri* as were two strains from scallop. Strains from scallop were also identified as *Shewanella putrefaciens* A (three strains), and *Shewanella*

putrefaciens D (five strains). The regional strains have been shown to be strains of *Vibrio* (Powell, 1978; Hollohan *et al.* 1986). These strains have been tested and retested (Martin-Kearley, 1992) and all were found to have fermentative metabolism. By using Biolog, some regional strains were assigned identities that could not be correct (eg. *Deleya marina*, *Pseudomonas stutzeri*, *Shewanella putrefaciens* A and D, and *Psychrobacter immobilis*), because these strains are obligate aerobes (Holt et al. 1994). It is important to note here that Fulthorpe and Wyndham (1991) also experienced difficulties with unknown strains being identified as *P. immobilis* although the cell shape and motility of the strains studied did not fit that of the unknown.

IV. A comparison of classifications based on Biolog and *Bergey's Manual* criteria.

Data consisting of a series of test results based on criteria described in Table 5.52A of the ninth edition of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994) were available for the regional and reference strains in this study. A dendrogram was produced based upon these "classical" test results. An examination of this dendrogram showed that

generally the reference strains clustered together. Most of the strains isolated from scallop clustered together and some clusters consisted almost entirely of strains isolated from *Alaria*. A further description of the classification of these strains was given by Martin-Kearley and Gow (1994) and Martin-Kearley *et al.* (1994). Before classifications based on Biolog and Bergey's criteria could be compared it was felt that the strains included in each analysis should be the same. To compare the dendrograms based on "classical' data and Biolog data a third dendrogram was produced. This dendrogram was similar to the one produced using classical data but with the addition of labels placed along each strain. This label indicated to which cluster each strain belonged in the dendrogram based on Biolog data. An examination of the new dendrogram indicated that there was a mixture of Biolog cluster membership within the clusters generated by Bergey's criteria. Based upon the labels, it appeared that the Bergey's based classification produced clusters in which members from the Biolog classification were intermixed. However, a closer look indicated some correlation between the two classifications. To determine whether or not there was a significant difference in the clusters generated from the Biolog data and those generated based on the Bergey's data a Pearson

Chi-Square test was performed. It was found that there was an association between the two dendrograms [p-value is < 0.01 (X^2= 119.91, DF = 25)]. Because these dendrograms were significantly dependent upon one another the results of one can probably predict the results of the other. The classification method that represents a picture closer to the "actual" relationship of these strains would have to be determined using other types of tests. These could include DNA based methods. Examples are 16S rRNA sequencing, DNA-DNA or RNA-DNA homology experiments, or polymerase chain reaction (PCR). An example of the use of PCR is given in the study of Martin-Kearley et al. (1994).

When the Biolog system is used in taxonomic studies the efficiency of the method would be an improvement over classical methods. Although the system appears to be inadequate for identification it has the potential to be a useful identification method if modified by the individual investigator. If an investigator is willing to generate her or his own databank of breathprints of reference cultures and to use an independent or alternative clustering method, then environmental strains can be compared with known species for similarity of metabolic features. This could be a useful contribution to our knowledge of the metabolic profiles of environmental bacteria and make comparative

studies between regions more meaningful than they are now.

In the final stages of preparing this manuscript a study based on the evaluation of the Biolog system for classification of marine bacteria was published. Rüger and Krambeck (1994) used the Biolog system to obtain metabolic profiles of 80 Arctic marine bacteria not included in the Biolog GN data base. These strains had already been characterized by growth experiments based on utilization of organic substrates as sole carbon and energy sources. Twenty-four of the carbon sources used in the conventional substrate utilization tests were the same as tests incorporated in the Biolog GN MicroPlates. This overlap allowed a comparison of the two test methods in order to determine if quickly obtained Biolog test results could be used to replace conventional test results. In general, the conventional substrate utilization tests gave more positive reactions than the Biolog technique. Cluster analysis performed on the marine strains, using the twenty-four tests, gave similar classifications at low similarity levels, but different classifications at higher similarity levels. This led Rüger and Krambeck (1994) to the conclusion that Biolog test results can lead to incorrect identifications of strains when they are used in place of conventional tests in taxonomic descriptions

presented in handbooks such as *Bergey's Manual of Systematic Bacteriology*. They believe this to be so because these classical descriptions are based on true utilization of organic substrates as opposed to oxidation of substrates. In the present study, classifications based on classical and Biolog methods were not significantly different although individual strains did not always appear in the same clusters by both methods. The Biolog system remains a very useful tool for the rapid differentiation of large numbers of strains by means of obtaining metabolic profiles, and the technique is useful for identification of unknown strains provided that reference or culture collection strains are included. Some additional basic tests may be useful. These may include a test for oxidative or fermentative metabolism.

V. General Conclusion:

The purpose of this study was to assess the Biolog system's capacity to identify and classify marine bacteria. The strains studied were *Vibrionaceae* reference cultures and strains isolated from a seasonally-cold ocean. To ensure that the *Vibrio*, *Listonella*, and *Photobacterium* type cultures were pure, classical test results (Farmer and Hickman-Brenner, 1992) were used in

conjunction with Biolog identifications.

It was found that, by using the Biolog system, 54% of the *Vibrionaceae* strains were correctly identified in approximately 24 hours when the Biolog procedure was followed. Under standard conditions 18% of the strains were incorrectly identified while 28% were not assigned an identity. Because marine bacteria require ions for solute retention the Biolog protocol was altered to bring these strains to a more optimal metabolic potential. It was found that 62% of strains could have been at their most metabolically active when suspended in a solution of marine cation supplement plus yeast. The second most effective suspending solution was the MCS which resulted in 24% of the strains having more positive wells than they would have had if they had been suspended in the standard 0.85% NaCl suspending solution. If identifications were accepted as correct when the similarity value was less than the normally acceptable (similarity <0.500) the percentage of correct identifications changed. Under these circumstances 56% were identified in the standard solution, 59% in MCS (formally 46%), and 54% in MCSpY (up from 41%). Although the MCSpY tended to support the most metabolic activity it was not the suspension solution of choice because the addition of yeast extract to the microplate may have

altered the very principle upon which Biolog is based. MCS was used to suspend the eighty regional strains because this solution encouraged high metabolic activity and had a comparable identification rate to the standard solution at 46%. It would be advantageous if Biolog would revise its data base to include marine strains that had been suspended in marine type salts.

The cultivation medium chosen was TSA plus glycerol with marine cation supplement diluted one in ten. There was no distinct advantage of using this medium over the Biolog Universal Growth Medium other than the benefits of working with a medium made in one's own laboratory. There is only one supplier of BUGM and competitive pricing is a factor that should be considered.

The majority of regional strains that were assigned a name by using the Biolog system probably were identified incorrectly if the type of metabolism was taken into consideration. That is, strains that were known to be facultatively anaerobic were assigned to genera that consisted of species with only oxidative metabolism. However, several regional strains were identified as *Vibrio* strains by the Biolog system.

A classification procedure based on data collected by using the Biolog system was not significantly different (p-value < 0.01) from a classification based on data produced from

traditional or classical bacteriological testing. This indicated that the Biolog microbial identification system can be an efficient method to collect data for future taxonomic studies. Further studies will be required to fully exploit the potential of the Biolog system as an identification and taxonomic tool.

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Appendix. Data matrices for the 80 regional strains and the *Vibrionaceae* reference strains used to construct dendrograms based on the determinative test results of *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994) [c:spsswin\lisa\bergey's.sav] and Biolog test results [c:\spsswin\lisa\biolog.sav].

	strain	swarming	pigment	arginine	oxidase	nitrate
1	1	0	0	0	1	0
2	2	0	0	0	1	0
3	3	0	0	0	1	0
4	4	0	0	0	1	0
5	5	0	0	0	1	0
6	6	0	0	0	1	0
7	7	0	0	0	1	0
8	8	0	0	0	1	0
9	9	0	0	0	1	0
10	10	0	0	0	1	0
11	11	0	0	0	1	0
12	12	0	0	0	1	0
13	13	0	0	0	1	0
14	14	0	0	0	1	0
15	15	0	0	0	1	0
16	16	0	0	0	1	0
17	17	0	0	0	1	1
18	18	0	0	0	1	1
19	19	0	0	0	1	1
20	20	0	0	0	1	1
21	21	0	0	1	1	1
22	22	0	0	1	1	1
23	23	0	0	1	1	1
24	24	0	0	1	1	1
25	25	0	0	1	1	1
26	26	0	0	1	1	1
27	27	0	0	1	1	1
28	28	0	0	1	1	1
29	29	0	0	0	1	1
30	30	0	0	0	1	1
31	31	0	0	0	1	1

	glu.gas	acetoin	temp.4	temp.30	temp.35	temp.40	chitin
1	0	0	1	1	0	0	0
2	0	0	1	1	0	0	0
3	0	0	1	1	0	0	0
4	0	0	1	1	1	0	0
5	0	0	1	1	0	0	0
6	0	0	1	1	0	0	0
7	0	0	1	1	0	0	0
8	0	0	1	1	0	0	0
9	0	0	1	1	0	0	0
10	0	0	1	1	0	0	0
11	0	0	1	1	0	0	0
12	0	0	1	1	1	1	0
13	0	0	1	1	1	0	0
14	0	0	1	1	0	0	0
15	0	0	1	1	0	0	0
16	0	0	1	1	0	0	0
17	0	0	1	1	1	0	0
18	0	0	1	1	1	0	0
19	0	0	1	1	1	0	0
20	0	0	1	1	1	1	0
21	0	0	1	1	0	0	1
22	0	0	1	1	0	0	1
23	0	0	1	1	0	0	0
24	0	0	1	1	0	0	1
25	0	0	1	1	0	0	0
26	0	0	1	1	0	0	1
27	0	0	1	1	1	0	1
28	0	0	1	0	0	0	1
29	0	0	1	0	0	0	0
30	0	0	1	1	0	0	1
31	0	0	1	1	0	0	1

	acetate	aconitat	b.alanin	d.alanin	l.alanin	y.a.buty	l.arabin
1	1	1	0	1	1	0	1
2	1	1	0	0	1	0	0
3	1	1	0	0	1	0	0
4	1	1	1	0	1	0	0
5	1	1	1	0	1	0	0
6	1	1	0	0	1	0	0
7	1	0	0	0	1	0	0
8	1	1	0	0	1	0	0
9	1	1	0	0	1	0	0
10	1	0	0	0	1	0	0
11	1	0	0	0	1	0	0
12	1	1	0	1	1	0	0
13	1	0	0	0	1	0	1
14	1	1	0	0	1	0	0
15	1	1	0	1	1	0	0
16	1	1	0	1	1	0	1
17	1	1	0	1	1	0	0
18	1	1	0	1	1	0	0
19	1	1	0	1	1	0	0
20	1	1	0	1	1	0	0
21	1	1	0	1	1	0	0
22	1	1	0	1	1	0	0
23	1	1	0	1	1	0	0
24	1	1	0	1	1	0	0
25	1	1	0	0	1	0	0
26	1	1	0	0	0	0	0
27	1	1	0	1	1	0	0
28	1	1	0	1	1	0	0
29	1	0	0	0	0	0	0
30	1	1	0	1	1	0	0
31	1	1	0	1	1	0	0

	l.aspart	caprate	caproate	caprylat	cellobio	citrate	citrulli
1	1	1	0	0	1	1	1
2	1	1	0	0	1	1	1
3	1	1	0	0	1	1	1
4	1	1	0	0	1	1	1
5	1	1	0	0	1	1	1
6	1	1	0	0	1	1	1
7	1	1	0	0	1	0	1
8	1	1	0	0	1	1	1
9	1	0	0	0	1	1	1
10	1	0	0	0	0	1	1
11	1	0	0	0	0	1	1
12	1	1	0	0	0	1	1
13	1	1	0	0	0	1	1
14	1	1	0	0	1	1	1
15	1	1	0	0	0	1	1
16	1	1	0	0	0	1	1
17	1	1	0	0	0	1	1
18	1	1	0	0	0	1	0
19	1	1	0	0	0	1	0
20	1	1	0	0	0	1	1
21	1	1	0	0	0	1	1
22	1	1	0	0	0	1	1
23	1	1	0	0	0	1	1
24	1	0	0	0	0	1	1
25	1	1	0	0	0	1	0
26	1	0	0	0	0	1	0
27	1	1	0	0	0	1	1
28	1	1	0	0	0	1	1
29	0	0	0	0	0	0	0
30	1	1	0	0	0	1	1
31	1	1	0	0	0	1	1

	ethanol	d.galact	d.gal.u	d.glucon	l.glutam	glutarat	d.glucur
1	0	1	0	1	1	0	1
2	0	1	0	1	1	0	0
3	0	1	0	1	1	0	0
4	1	1	0	1	1	1	0
5	0	1	0	1	1	0	0
6	0	1	0	1	1	0	0
7	1	1	0	1	1	0	0
8	0	1	0	1	1	0	0
9	1	1	0	0	1	0	0
10	1	1	0	0	1	0	0
11	1	1	0	0	1	0	0
12	1	1	0	0	1	0	0
13	1	1	0	1	1	0	0
14	1	1	0	1	1	0	0
15	1	1	0	0	1	0	0
16	1	1	1	0	1	1	1
17	1	1	0	0	1	0	0
18	0	1	0	0	1	0	1
19	0	1	0	0	1	0	1
20	0	1	0	0	1	0	1
21	0	1	0	0	1	0	1
22	0	1	0	0	1	0	0
23	0	1	0	0	1	0	0
24	0	1	0	0	1	0	0
25	0	1	0	0	1	0	0
26	0	1	0	0	1	0	0
27	0	1	0	0	1	0	1
28	0	1	0	0	1	0	1
29	0	1	0	0	1	0	1
30	0	1	0	0	1	0	0
31	0	1	0	0	1	0	0

	dl.glyce	glycine	heptanoa	l.histid	p.hydrox	b.hydrox	inositol
1	0	1	1	0	0	0	1
2	0	0	1	0	0	0	1
3	1	1	1	0	0	0	0
4	1	1	1	1	1	1	0
5	0	1	1	0	0	0	0
6	0	1	1	0	0	0	0
7	0	1	1	0	0	0	0
8	0	1	1	0	0	0	0
9	0	1	1	0	1	0	0
10	0	1	1	0	1	0	0
11	0	1	0	1	1	0	0
12	1	0	1	1	1	0	1
13	0	0	0	1	1	0	1
14	0	0	1	1	1	0	1
15	1	1	0	1	1	0	1
16	1	1	1	1	1	1	1
17	0	1	0	1	1	0	1
18	1	1	0	1	0	0	0
19	1	1	0	1	0	0	0
20	1	1	0	1	0	0	0
21	1	1	1	1	0	0	0
22	1	0	0	1	0	0	0
23	1	1	0	1	0	0	0
24	1	1	0	1	0	0	0
25	1	0	1	1	0	0	0
26	0	0	0	1	0	0	0
27	1	1	0	1	0	0	0
28	1	1	0	1	0	0	0
29	1	0	0	0	0	0	0
30	1	1	0	1	0	0	0
31	1	1	0	1	0	0	0

	isobutyr	a.ketogl	dl.lacta	lactose	l.leucin	dl.malat	d.mannit
1	0	0	1	1	1	1	1
2	0	0	0	1	1	0	1
3	0	0	0	1	1	0	1
4	0	0	1	1	1	0	1
5	0	0	0	1	1	0	1
6	0	0	0	1	1	0	1
7	0	0	0	1	1	0	1
8	0	0	0	1	1	0	1
9	0	0	0	1	1	0	1
10	0	0	0	1	1	0	1
11	0	0	0	1	1	0	1
12	0	0	0	1	1	0	1
13	0	0	0	1	1	0	1
14	0	0	0	1	1	0	1
15	0	0	0	1	1	1	1
16	1	0	1	1	1	1	1
17	0	0	1	1	1	0	1
18	0	0	1	1	1	0	1
19	0	0	1	1	1	0	1
20	0	0	1	1	1	0	1
21	0	0	1	1	1	0	1
22	0	0	1	1	1	0	1
23	0	0	1	1	1	0	1
24	0	0	1	1	1	0	1
25	0	0	1	1	1	0	1
26	0	0	1	1	1	0	1
27	0	0	1	1	1	0	1
28	0	0	1	1	1	0	1
29	0	0	0	0	1	0	0
30	0	0	1	1	1	0	1
31	0	0	1	1	0	0	1

	d.mannos	melibios	l.ornith	pelargon	l.prolin	propanol	propiona
1	1	1	1	1	1	1	1
2	1	1	1	1	1	0	1
3	1	1	1	1	1	0	1
4	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1
6	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1
8	1	1	1	1	1	0	1
9	1	1	1	0	1	1	1
10	1	1	1	0	1	1	1
11	1	1	1	0	1	1	1
12	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1
17	1	1	0	0	1	1	1
18	1	0	0	0	1	0	1
19	1	1	0	0	1	0	1
20	1	0	1	0	1	0	1
21	1	0	1	0	1	0	1
22	1	0	1	0	1	0	1
23	1	1	0	0	1	0	1
24	1	0	1	0	1	0	1
25	1	0	0	0	1	0	1
26	1	0	0	0	1	0	1
27	1	0	0	0	1	0	1
28	1	0	1	0	1	0	1
29	1	0	0	0	0	0	1
30	1	0	0	0	1	0	1
31	1	0	1	0	1	0	1

	putresci	pyruvate	quinate	l.rhamno	d.ribose	salicin	l.serine
1	0	1	0	0	0	1	1
2	0	1	0	0	0	0	1
3	0	1	0	0	0	0	1
4	0	1	1	1	1	0	1
5	0	1	0	0	0	0	1
6	0	1	0	0	0	0	1
7	0	1	0	0	0	0	1
8	0	1	0	0	0	0	1
9	0	1	0	0	0	0	1
10	0	1	0	0	0	0	1
11	0	1	0	0	1	0	1
12	0	1	0	1	1	1	1
13	0	1	0	1	1	1	1
14	0	1	0	1	1	1	1
15	0	1	0	1	1	1	1
16	0	1	1	1	1	1	1
17	0	1	0	1	1	1	1
18	0	1	0	0	1	0	1
19	0	1	0	0	1	0	1
20	0	1	0	0	1	0	1
21	0	1	0	0	1	0	1
22	0	1	0	0	1	0	1
23	0	1	0	0	1	0	1
24	0	1	0	0	1	0	1
25	0	1	0	0	1	0	1
26	0	1	0	0	1	0	1
27	0	1	0	0	1	0	1
28	0	1	0	0	1	0	1
29	0	1	0	0	1	0	0
30	0	1	0	0	1	0	1
31	0	1	0	0	1	0	1

	d.sorbit	sucrose	trehalos	l.tyrosi	valerate	d.xylose	amylase
1	1	1	1	1	1	1	0
2	1	1	1	1	1	1	0
3	0	1	1	1	1	1	1
4	1	1	1	1	1	1	0
5	0	1	0	1	1	1	0
6	0	1	1	1	1	1	0
7	1	1	1	1	1	1	1
8	0	1	0	1	1	1	0
9	0	1	1	1	1	0	0
10	0	0	1	1	1	1	0
11	0	0	1	1	1	1	0
12	1	1	1	1	0	0	0
13	1	1	1	1	0	1	0
14	1	1	1	1	1	1	1
15	1	0	1	1	0	1	0
16	1	0	1	1	1	1	0
17	1	0	1	0	0	1	0
18	0	0	1	0	0	0	0
19	0	0	1	0	0	0	0
20	0	0	1	0	0	0	0
21	0	0	1	1	1	0	1
22	0	0	1	0	0	0	1
23	0	0	1	0	0	0	0
24	0	0	1	0	0	0	1
25	0	0	1	1	1	0	0
26	0	0	1	0	0	0	1
27	0	0	1	0	0	0	1
28	0	0	1	0	0	0	1
29	0	0	1	0	0	0	1
30	0	0	1	1	0	0	1
31	0	0	1	0	0	0	0

	gelatin	lipase	alginase
1	0	0	1
2	1	1	0
3	0	0	1
4	1	1	1
5	0	0	1
6	0	0	1
7	0	0	1
8	1	1	0
9	0	0	1
10	0	0	1
11	0	0	1
12	0	0	1
13	1	1	0
14	1	1	0
15	1	1	0
16	0	1	1
17	0	0	1
18	0	0	1
19	0	0	1
20	1	1	0
21	1	1	1
22	1	1	0
23	0	1	1
24	1	1	0
25	0	0	1
26	1	1	1
27	1	1	0
28	1	1	0
29	1	1	1
30	1	1	1
31	1	1	0

	strain	swarming	pigment	arginine	oxidase	nitrate
32	32	0	0	1	1	1
33	33	0	0	1	1	1
34	34	0	0	1	1	0
35	35	0	0	1	1	0
36	36	0	0	1	1	1
37	37	0	0	0	1	1
38	38	0	0	0	1	1
39	39	0	0	0	1	1
40	40	0	0	0	1	1
41	41	0	0	0	1	1
42	42	0	0	0	1	1
43	43	0	0	0	1	1
44	44	0	0	0	1	1
45	45	0	0	0	1	1
46	46	0	0	0	1	1
47	47	0	0	0	1	0
48	48	0	0	0	1	0
49	49	0	0	0	1	0
50	50	0	0	0	1	0
51	51	0	0	0	1	0
52	52	0	0	0	1	0
53	53	0	0	0	1	1
54	54	0	0	1	1	1
55	55	0	0	0	1	0
56	56	0	0	0	1	0
57	57	0	0	1	1	1
58	58	0	0	1	1	1
59	59	0	0	0	1	1
60	60	0	0	0	1	1
61	61	0	0	0	1	1
62	62	0	0	1	1	1

	glu.gas	acetoin	temp.4	temp.30	temp.35	temp.40	chitin
32	0	0	1	1	0	0	0
33	0	0	1	1	0	0	0
34	0	0	1	0	0	0	0
35	0	0	1	0	0	0	1
36	0	0	1	0	0	0	1
37	0	0	1	0	0	0	0
38	0	0	1	1	0	0	1
39	0	0	1	1	0	0	1
40	0	0	1	1	0	0	0
41	0	0	1	1	0	0	0
42	0	0	1	1	1	1	0
43	0	0	1	1	1	1	0
44	0	0	1	1	0	0	0
45	0	0	1	1	0	0	1
46	0	0	1	1	0	0	0
47	0	0	1	1	1	0	0
48	0	0	1	1	1	1	0
49	0	0	1	1	0	0	0
50	0	0	1	1	1	1	0
51	0	0	1	1	0	0	0
52	0	0	1	1	1	0	0
53	0	0	1	1	1	1	0
54	0	0	1	0	0	0	1
55	0	0	1	1	0	0	0
56	0	0	1	1	1	1	0
57	0	0	1	1	1	1	1
58	0	0	1	0	0	0	1
59	0	0	1	0	0	0	0
60	0	0	1	0	0	0	0
61	0	0	1	1	1	1	0
62	0	0	1	1	0	0	1

	acetate	aconitat	b.alanin	d.alanin	l.alanin	y.a.buty	l.arabin
32	1	1	0	1	1	0	0
33	1	1	0	1	1	0	0
34	0	0	0	0	0	0	0
35	1	1	0	0	1	0	0
36	1	1	0	1	1	0	0
37	1	1	0	1	1	0	0
38	1	0	0	0	1	0	0
39	1	0	0	0	1	0	0
40	1	0	0	0	1	0	0
41	1	0	0	0	1	0	0
42	1	0	0	0	1	0	0
43	1	0	0	0	1	0	0
44	1	0	0	0	1	0	0
45	1	0	0	0	1	0	0
46	1	1	0	0	1	0	0
47	1	1	1	1	1	1	0
48	1	1	1	1	1	1	0
49	1	1	1	1	1	1	0
50	1	1	1	1	1	1	0
51	1	1	0	1	1	0	0
52	1	1	0	1	1	1	0
53	1	1	1	1	1	1	0
54	1	1	0	1	1	0	0
55	1	1	0	1	1	1	0
56	1	1	1	1	1	1	1
57	1	1	1	1	1	1	1
58	1	1	0	1	1	0	0
59	1	1	0	1	1	0	0
60	1	1	0	1	1	0	0
61	1	1	0	1	1	0	0
62	1	1	0	1	1	0	0

	l.aspart	caprate	caproate	caprylat	cellobio	citrate	citrulli
32	1	1	0	0	0	1	1
33	1	1	0	0	0	1	1
34	0	0	0	0	0	1	0
35	1	0	0	0	0	0	0
36	1	1	0	0	0	1	1
37	1	0	0	0	0	1	1
38	0	1	0	0	0	0	0
39	0	1	0	0	0	0	0
40	0	1	0	0	0	0	0
41	0	1	0	0	1	0	0
42	0	1	0	0	1	0	1
43	0	1	0	0	1	0	0
44	0	1	0	0	0	0	0
45	0	1	0	0	0	0	0
46	0	1	0	1	1	0	0
47	1	1	0	1	1	1	1
48	1	1	0	1	1	1	1
49	1	1	0	1	1	1	1
50	1	1	0	1	1	1	1
51	1	1	0	1	1	1	1
52	1	1	0	1	1	1	1
53	1	1	0	0	1	1	1
54	1	1	0	0	1	1	1
55	1	0	0	0	1	1	1
56	1	1	0	0	1	1	0
57	1	1	0	0	1	1	1
58	1	1	0	0	1	1	1
59	1	1	0	0	1	1	1
60	1	1	0	0	1	1	0
61	1	1	0	0	1	1	1
62	1	0	0	0	1	1	1

	ethanol	d.galact	d.gal.u	d.glucon	l.glutam	glutarat	d.glucur
32	0	1	0	0	1	0	0
33	1	1	0	0	1	0	0
34	0	1	0	0	1	0	0
35	0	0	0	0	1	0	0
36	0	1	0	0	1	0	0
37	0	1	0	0	1	0	1
38	0	0	0	0	1	0	0
39	0	0	0	0	1	0	0
40	0	0	0	0	1	0	0
41	0	0	0	0	1	0	0
42	0	0	0	0	1	0	0
43	0	0	0	0	1	0	0
44	0	0	0	0	1	0	0
45	0	0	0	0	1	0	0
46	0	0	0	0	1	0	0
47	1	1	0	1	1	0	0
48	1	1	1	1	1	0	1
49	1	1	0	1	1	1	0
50	1	1	0	1	1	0	0
51	0	1	0	1	1	0	0
52	0	1	0	1	1	0	0
53	1	1	0	1	1	0	1
54	0	1	0	1	1	0	1
55	0	1	0	1	1	0	0
56	1	1	0	1	1	0	1
57	1	1	0	1	1	0	1
58	0	1	0	1	1	0	1
59	1	1	0	1	1	0	1
60	0	1	0	1	1	0	0
61	0	1	0	0	1	0	1
62	0	1	0	0	1	0	1

	dl.glyce	glycine	heptanoa	l.histid	p.hydrox	b.hydrox	inositol
32	1	1	0	1	0	0	0
33	1	1	0	1	0	0	0
34	1	0	0	1	0	0	0
35	1	0	0	0	0	0	0
36	1	1	0	1	0	0	0
37	1	1	0	1	0	0	0
38	1	0	0	0	0	0	0
39	1	0	0	0	0	0	0
40	1	0	0	0	0	0	0
41	1	0	0	0	0	0	0
42	1	0	0	0	0	0	0
43	1	0	0	0	0	0	0
44	1	0	1	0	0	0	0
45	1	0	1	0	0	0	0
46	1	0	1	0	0	1	0
47	1	1	1	1	0	0	1
48	1	1	1	0	0	1	1
49	1	1	1	1	0	1	0
50	1	1	1	0	0	1	1
51	0	1	1	0	0	0	0
52	1	1	1	0	0	0	0
53	1	1	1	1	0	1	1
54	1	1	0	1	0	0	0
55	1	0	0	0	0	0	0
56	1	1	1	1	0	1	1
57	1	1	1	1	0	1	1
58	1	1	0	1	0	0	0
59	1	1	0	1	0	0	0
60	1	1	0	1	0	0	0
61	1	1	0	1	0	0	0
62	1	1	0	1	0	0	0

	isobutyr	a.ketogl	dl.lacta	lactose	l.leucin	dl.malat	d.mannit
32	0	0	1	1	1	0	1
33	0	0	1	1	1	0	1
34	0	0	0	1	0	0	1
35	0	0	1	0	0	0	0
36	0	0	1	1	1	0	1
37	0	0	1	1	1	0	1
38	0	0	1	0	0	0	1
39	0	0	1	0	0	0	1
40	0	0	1	0	0	0	1
41	0	0	1	0	1	0	1
42	0	0	1	0	1	0	0
43	1	0	1	0	1	0	0
44	0	0	1	0	0	0	1
45	0	0	1	0	0	0	1
46	0	0	1	0	1	0	1
47	1	0	0	1	1	1	1
48	0	0	1	1	1	1	1
49	1	0	1	1	1	1	1
50	0	0	1	1	1	1	1
51	0	0	0	1	1	0	1
52	1	0	0	1	1	0	1
53	0	0	1	1	1	1	1
54	0	0	1	1	1	0	1
55	0	0	0	0	1	0	1
56	0	0	1	1	1	1	1
57	0	0	1	1	0	1	1
58	0	0	1	1	1	0	1
59	0	0	1	1	0	0	1
60	0	0	1	1	0	0	1
61	0	0	1	1	0	0	1
62	0	0	1	1	0	0	1

	d.mannos	melibios	l.ornith	pelargon	l.prolin	propanol	propiona
32	1	0	1	0	1	0	1
33	1	0	0	0	1	1	1
34	1	0	0	0	1	1	1
35	0	0	0	0	0	0	1
36	1	1	0	0	1	0	1
37	1	1	1	0	1	0	1
38	0	0	1	0	0	0	1
39	0	0	1	0	0	0	1
4(	0	0	1	0	0	0	1
41	0	0	1	0	0	0	1
42	0	0	1	0	0	0	1
43	0	0	1	0	0	0	1
44	0	0	1	0	0	0	1
45	0	0	1	0	0	0	1
46	0	0	1	1	0	0	1
47	1	1	1	1	1	1	1
48	1	1	1	1	1	1	1
49	1	1	1	1	1	1	1
50	1	1	1	1	1	1	1
51	1	1	0	1	1	1	1
52	1	1	0	1	1	1	1
53	1	1	1	1	1	1	1
54	1	0	0	0	1	0	1
55	1	1	0	0	0	0	1
56	1	1	1	1	1	1	1
57	1	1	1	1	1	1	1
58	1	1	1	0	1	0	1
59	1	0	1	0	1	0	1
60	1	0	0	1	1	0	1
61	1	0	0	0	1	0	1
62	1	0	1	0	1	0	1

	putresci	pyruvate	quinate	l.rhamno	d.ribose	salicin	l.serine
32	0	1	0	0	1	0	1
33	0	1	0	0	1	0	1
34	0	1	0	0	1	0	0
35	0	1	0	0	0	0	0
36	0	1	0	0	1	0	1
37	0	1	0	0	1	0	1
38	0	1	0	0	1	0	1
39	0	1	0	0	1	0	1
40	0	1	0	0	1	0	1
41	1	1	0	0	0	0	1
42	1	1	0	0	0	0	1
43	1	1	0	0	0	0	1
44	1	1	0	0	0	0	1
45	0	1	0	0	0	0	1
46	0	1	0	0	0	0	1
47	0	1	0	1	1	0	1
48	1	1	0	1	1	0	1
49	1	1	0	0	0	0	1
50	0	1	0	0	1	0	1
51	0	1	0	0	0	0	1
52	0	1	0	0	0	0	1
53	0	1	0	0	1	0	1
54	0	1	0	0	1	0	1
55	0	1	0	0	1	0	1
56	0	1	0	1	1	1	1
57	1	1	0	0	1	1	1
58	0	1	0	0	1	0	1
59	0	1	0	0	1	0	1
60	0	1	0	0	1	0	1
61	0	1	0	0	1	0	1
62	0	1	0	0	1	0	1

	d.sorbit	sucrose	trehalos	l.tyrosi	valerate	d.xylose	amylase
32	0	0	1	1	0	0	0
33	0	0	1	0	0	0	0
34	0	0	1	0	0	0	0
35	0	0	0	0	0	0	1
36	0	0	1	0	0	0	1
37	0	0	1	1	0	1	0
38	0	0	0	0	0	0	0
39	0	0	0	0	0	1	1
40	0	0	0	0	0	1	0
41	0	1	0	0	0	0	0
42	0	1	0	0	0	0	0
43	0	1	0	0	0	0	0
44	0	1	0	0	1	0	0
45	0	1	0	0	1	0	1
46	0	1	0	0	1	0	1
47	1	1	0	1	1	1	1
48	0	1	0	1	1	1	0
49	0	1	0	1	1	1	0
50	0	0	0	1	1	1	0
51	0	1	0	1	1	1	0
52	0	1	0	1	1	1	0
53	0	0	1	1	1	0	0
54	0	0	1	0	0	0	0
55	0	0	1	1	0	0	0
56	0	0	1	1	1	0	0
57	0	1	1	1	1	1	1
58	0	0	1	1	0	0	1
59	0	0	1	1	0	0	0
60	0	0	1	1	0	0	0
61	0	0	1	1	0	0	0
62	0	0	1	1	0	0	1

	gelatin	lipase	alginase
32	0	0	1
33	0	0	1
34	0	0	1
35	1	1	0
36	1	1	0
37	0	0	1
38	0	0	1
39	1	1	0
40	0	0	1
41	0	0	1
42	1	0	1
43	0	1	1
44	0	1	1
45	1	1	1
46	1	1	1
47	1	1	0
48	0	0	0
49	0	1	1
50	0	1	1
51	1	1	0
52	0	0	1
53	0	1	0
54	0	1	0
55	0	0	0
56	0	0	1
57	1	1	0
58	1	1	1
59	1	1	0
60	0	1	1
61	0	1	0
62	1	1	1

	strain	swarming	pigment	arginine	oxidase	nitrate
63	63	0	0	1	1	1
64	64	0	0	1	1	1
65	65	0	0	1	1	1
66	66	0	0	0	1	1
67	67	0	0	0	1	1
68	68	0	0	0	1	0
69	69	0	0	0	1	1
70	70	0	0	0	1	1
71	71	0	0	0	1	1
72	72	0	0	0	1	0
73	73	0	0	0	1	0
74	74	0	0	0	1	1
75	75	0	0	0	1	1
76	76	0	0	0	1	1
77	77	0	0	0	1	1
78	78	0	0	0	1	1
79	79	0	0	0	1	1
80	80	0	0	0	1	1
81	L. damsela	0	0	0	1	1
82	V. fisheri	0	0	0	1	1
83	V. costicola	0	0	1	1	1
84	V. metschnikovii	0	0	0	0	1
85	V. natriegens	0	0	0	1	1
86	V. proteolyticus	1	0	1	1	1
87	V. marinus	0	0	0	1	1
88	A. caviae	0	0	1	1	1
89	V alginolyticus	1	0	0	1	1
90	V. parahaemo.	0	0	0	1	1
91	V. [illegible]ibiashii	1	0	1	1	1
92	L. anguillarum	0	0	1	1	1
93	A. hydrophilia	0	0	1	1	1

	glu.gas	acetoin	temp.4	temp.30	temp.35	temp.40	chitin
63	0	0	1	1	0	0	1
64	0	0	1	0	0	0	1
65	0	0	1	0	0	0	1
66	0	0	1	1	0	0	1
67	0	0	1	1	0	0	0
68	0	0	1	1	0	0	0
69	0	0	1	1	0	0	1
70	0	0	1	1	0	0	0
71	0	0	1	1	0	0	0
72	0	0	1	1	0	0	0
73	0	0	1	1	1	1	0
74	0	0	1	1	1	1	0
75	0	0	1	1	1	1	0
76	0	0	1	1	1	1	0
77	0	0	1	1	1	1	0
78	0	0	1	1	1	1	0
79	0	0	1	1	1	0	0
80	0	0	1	1	0	0	0
81	1	0	0	1	1	0	0
82	0	0	0	1	0	0	0
83	0	0	0	1	1	0	0
84	0	1	1	1	1	1	0
85	0	0	0	1	1	1	0
86	1	1	0	1	1	1	1
87	0	0	1	0	0	0	1
88	0	0	1	1	1	1	1
89	0	1	0	1	1	1	1
90	0	0	0	1	1	1	0
91	0	0	0	1	1	1	1
92	0	1	1	1	1	0	1
93	0	1	1	1	1	1	1

	acetate	aconitat	b.alanin	d.alanin	l.alanin	y.a.buty	l.arabin
63	1	1	0	1	1	0	0
64	1	1	0	1	1	0	0
65	1	1	0	1	1	0	0
66	1	0	0	1	1	0	0
67	1	0	0	1	1	0	0
68	1	0	0	1	1	1	0
69	1	0	0	0	1	0	0
70	1	0	0	0	1	0	0
71	1	1	0	0	1	0	1
72	1	0	0	1	1	1	1
73	1	1	1	1	1	0	1
74	1	1	1	1	1	1	1
75	1	1	1	1	1	1	1
76	1	1	1	0	1	0	1
77	1	1	1	1	1	1	1
78	1	1	0	1	1	0	1
79	1	1	0	0	1	0	1
80	1	1	0	0	0	0	0
81	1	0	1	1	1	1	1
82	1	0	0	0	0	0	0
83	1	0	0	1	1	1	1
84	1	0	0	1	1	0	0
85	1	0	1	1	1	1	1
86	1	1	1	1	1	0	0
87	1	0	0	1	0	0	0
88	1	0	0	1	1	1	1
89	1	0	0	1	1	0	1
90	1	0	0	1	1	0	1
91	1	1	1	1	1	0	0
92	1	0	0	1	1	0	1
93	1	0	0	1	1	1	1

	l.aspart	caprate	caproate	caprylat	cellobio	citrate	citrulli
63	1	1	0	0	1	1	1
64	1	1	0	0	1	1	1
65	1	1	0	0	1	1	1
66	1	1	0	0	1	0	1
67	1	1	0	0	1	0	0
68	1	0	0	0	0	0	0
69	0	1	0	0	1	0	0
70	0	1	0	0	1	0	0
71	0	1	0	0	1	0	0
72	0	1	0	0	0	0	0
73	0	1	0	0	0	0	1
74	0	1	0	0	0	1	1
75	0	1	0	0	0	1	1
76	0	1	0	0	1	1	1
77	0	1	0	0	1	1	1
78	1	1	0	0	0	1	0
79	1	1	0	0	0	1	0
80	1	1	0	0	0	0	0
81	1	1	1	1	0	1	1
82	0	0	1	1	1	0	0
83	1	1	1	1	0	1	1
84	1	1	1	1	0	1	1
85	1	1	1	1	0	1	1
86	1	1	1	1	0	1	1
87	1	1	0	0	0	0	0
88	1	1	1	1	1	1	0
89	1	1	0	1	0	1	0
90	1	1	0	1	0	1	0
91	1	1	1	1	1	1	1
92	1	1	0	1	1	0	1
93	1	1	1	1	0	1	0

	ethanol	d.galact	d.gal.u	d.glucon	l.glutam	glutarat	d.glucur
63	0	1	0	0	1	0	0
64	0	1	0	1	1	0	0
65	0	1	0	0	1	0	0
66	0	1	0	0	1	0	0
67	0	1	0	1	1	0	0
68	0	1	0	0	1	1	0
69	0	0	0	0	1	0	0
70	0	1	0	0	1	0	0
71	0	0	0	0	1	0	1
72	1	1	0	0	1	1	1
73	1	1	0	1	1	1	1
74	1	1	0	1	1	1	1
75	1	1	0	1	1	1	1
76	1	1	0	0	1	1	1
77	1	1	0	1	1	1	1
78	0	1	0	1	1	1	0
79	0	1	0	0	1	1	0
80	0	0	0	0	1	0	0
81	1	0	1	0	1	1	1
82	0	0	0	0	0	0	0
83	1	0	1	1	1	1	1
84	0	0	0	1	1	1	0
85	1	1	0	1	1	1	0
86	0	0	0	1	1	0	0
87	0	1	0	1	1	0	0
88	0	0	0	1	1	0	0
89	0	1	0	0	1	0	0
90	1	1	0	1	1	0	0
91	1	0	0	1	1	0	0
92	0	0	0	1	1	0	0
93	0	1	0	1	1	1	0

	dl.glyce	glycine	heptanoa	l.histid	p.hydrox	b.hydrox	inositol
63	1	1	0	1	0	0	0
64	1	1	0	1	0	0	0
65	1	1	0	1	0	0	0
66	1	1	0	0	0	0	0
67	1	0	1	0	0	0	0
68	0	0	1	1	0	1	0
69	1	0	1	0	0	0	0
70	1	0	0	0	0	0	0
71	1	0	0	0	0	1	0
72	0	0	1	1	0	1	1
73	1	0	1	1	0	1	0
74	1	1	1	1	0	1	0
75	1	1	1	1	0	1	0
76	1	1	1	1	1	1	1
77	1	1	1	1	1	1	1
78	1	1	1	0	0	1	0
79	1	0	1	1	0	1	0
80	1	0	0	0	0	0	0
81	1	1	1	1	0	1	0
82	0	0	1	0	1	1	1
83	1	1	1	1	0	1	0
84	0	0	1	1	0	1	1
85	1	1	1	1	1	1	0
86	1	1	1	1	0	0	0
87	0	0	0	0	0	0	0
88	1	0	1	1	0	1	0
89	1	1	1	1	0	0	0
90	1	1	1	1	0	0	0
91	1	1	1	1	0	0	0
92	1	0	1	0	0	0	0
93	1	0	1	1	0	1	0

	isobutyr	a.ketogl	dl.lacta	lactose	l.leucin	dl.malat	d.mannit
63	0	0	1	1	0	0	1
64	0	0	1	1	0	0	1
65	0	0	1	1	0	0	1
66	0	0	1	0	1	0	1
67	1	0	1	0	1	0	1
68	0	0	1	0	1	0	1
69	1	0	1	0	1	0	1
70	0	0	1	0	1	0	1
71	0	0	1	0	1	0	1
72	1	0	1	0	1	0	0
73	1	0	1	0	1	0	0
74	1	0	1	0	1	0	1
75	1	0	1	0	1	0	1
76	1	0	1	0	1	0	1
77	1	0	1	0	1	0	1
78	0	0	1	0	1	0	1
79	0	0	1	0	0	0	1
80	0	0	1	0	0	0	0
81	1	0	1	1	1	1	1
82	1	0	1	0	0	0	0
83	1	0	1	0	1	1	1
84	1	0	1	0	1	1	1
85	1	0	1	0	1	1	1
86	0	0	1	0	1	1	1
87	0	1	1	0	0	1	0
88	1	0	1	0	1	1	1
89	0	0	1	0	1	1	1
90	0	0	1	0	1	1	1
91	0	0	1	0	1	1	1
92	0	0	1	1	0	1	1
93	1	0	1	1	1	1	1

	d.mannos	melibios	l.ornith	pelargon	l.prolin	propanol	propiona
63	1	1	1	0	1	0	1
64	1	0	0	0	1	0	1
65	1	1	1	0	1	0	1
66	1	0	1	0	1	0	1
67	1	0	0	0	1	0	1
68	1	0	0	0	1	0	1
69	1	0	0	0	1	0	1
70	0	0	0	0	0	0	1
71	0	0	0	0	0	0	1
72	0	1	0	0	1	0	1
73	0	1	1	0	1	0	1
74	0	1	1	1	1	1	1
75	1	1	0	1	1	1	1
76	1	1	0	0	1	1	1
77	1	1	1	1	1	1	1
78	0	0	0	1	1	0	1
79	0	0	0	0	0	0	1
80	0	0	0	0	0	0	1
81	1	0	1	1	1	1	1
82	0	1	0	1	0	0	0
83	0	0	1	1	1	1	1
84	1	0	1	1	1	0	1
85	1	0	1	1	1	1	1
86	1	0	1	1	1	1	1
87	0	0	0	0	1	0	0
88	0	0	1	1	1	0	1
89	0	0	1	1	1	1	1
90	1	0	1	1	1	1	1
91	1	0	1	1	1	1	1
92	1	0	0	0	1	0	0
93	1	0	0	1	1	0	1

	putresci	pyruvate	quinate	l.rhamno	d.ribose	salicin	l.serine
63	0	1	0	0	1	1	1
64	0	1	0	0	1	0	1
65	0	1	0	0	1	0	1
66	1	1	0	0	1	0	1
67	1	1	0	0	1	0	1
68	0	1	0	0	1	0	1
69	1	1	0	0	1	0	1
70	1	1	0	0	0	0	1
71	1	1	0	0	0	0	1
72	1	1	0	0	1	0	1
73	0	1	0	0	1	0	1
74	1	1	0	0	1	0	0
75	1	1	0	0	1	0	0
76	1	1	0	1	1	1	1
77	1	1	0	1	1	1	1
78	0	1	1	0	0	0	0
79	0	1	1	0	0	0	1
80	0	1	1	0	0	0	1
81	1	1	0	1	1	1	1
82	0	0	0	0	0	0	0
83	1	1	0	1	1	1	1
84	0	1	0	0	1	1	1
85	1	1	1	1	1	1	1
86	1	1	0	0	1	1	1
87	0	0	0	0	1	0	1
88	1	1	0	0	1	1	1
89	1	1	0	1	1	1	1
90	1	1	0	0	1	1	1
91	1	1	0	0	1	1	1
92	0	1	0	0	1	0	1
93	1	1	0	0	1	1	1

	d.sorbit	sucrose	trehalos	l.tyrosi	valerate	d.xylose	amylase
63	0	0	1	1	0	0	1
64	0	1	1	1	0	0	1
65	0	1	1	0	0	0	1
66	0	1	0	1	0	0	0
67	0	1	0	1	1	0	1
68	0	0	1	1	1	0	1
69	0	1	0	0	1	0	1
70	0	1	0	1	1	0	1
71	0	1	1	1	1	1	1
72	0	0	0	1	1	1	0
73	0	0	1	1	1	1	0
74	0	1	1	1	1	1	0
75	0	1	1	1	1	1	0
76	1	1	1	1	1	1	1
77	1	1	1	1	1	1	0
78	0	0	1	1	1	1	1
79	0	0	0	0	1	1	1
80	0	1	0	0	1	0	1
81	1	1	1	1	1	0	1
82	0	0	0	0	1	1	1
83	1	1	1	1	1	0	0
84	0	1	1	1	1	0	1
85	0	1	1	1	1	0	1
86	1	1	1	1	0	0	1
87	0	0	0	0	0	0	1
88	0	1	1	1	1	0	1
89	0	1	1	1	0	0	1
90	1	1	1	0	0	0	1
91	1	1	1	1	0	0	1
92	1	1	1	0	0	0	1
93	1	1	1	1	1	0	1

	gelatin	lipase	alginase
63	1	1	1
64	0	1	1
65	0	1	0
66	1	1	1
67	1	1	1
68	1	1	1
69	1	1	1
70	1	1	1
71	1	1	1
72	0	1	1
73	0	1	0
74	0	1	0
75	0	1	0
76	1	1	1
77	0	1	0
78	0	1	0
79	0	1	0
80	1	1	1
81	0	0	0
82	0	0	0
83	1	0	0
84	1	1	0
85	1	1	0
86	1	1	0
87	1	1	0
88	1	1	0
89	1	1	0
90	1	1	0
91	1	1	0
92	1	1	0
93	1	1	0

	strain	swarming	pigment	arginine	oxidase	nitrate
94	V. splendidus	0	0	1	1	0
95	L. pelagia	0	0	1	1	1
96	V. nereis	1	0	1	1	1
97	V. harveyi	0	0	0	1	1
98	V. campbellii	0	0	0	1	1
99	A. salmonicida	0	0	1	1	0
100	V. nigrapulchritudo	0	0	0	1	1
101	V. vulnificus	0	0	0	1	1
102	V. gazogenes	0	1	0	0	0
103	V. diazotrophicus	0	0	1	1	1
104	V. ordalii	0	0	0	1	0
105	V. hollisae	0	0	0	1	1
106	V. mimicus	0	0	0	1	1
107	V. fluvialis	0	0	0	1	1
108	V. orientalis	0	0	1	1	1
109	V. furnissii	0	0	1	1	1
110	V. aestuarianus	0	0	1	1	1
111	V. carchariae	1	0	0	1	1
112	V. cincinnatiensis	0	0	0	1	1
113	V. mediterranei	0	0	1	1	1
114	A. sobria	0	0	1	1	1
115		.	.	.	.	.
116		.	.	.	.	.
117		.	.	.	.	.
118		.	.	.	.	.
119		.	.	.	.	.
120		.	.	.	.	.
121		.	.	.	.	.

	glu.gas	acetoin	temp.4	temp.30	temp.35	temp.40	chitin
94	0	0	0	1	0	0	1
95	0	0	0	1	1	1	0
96	0	0	0	1	1	1	1
97	0	0	0	1	1	0	0
98	0	0	0	1	1	0	1
99	0	1	1	1	1	0	1
100	0	0	0	1	1	0	0
101	0	0	0	1	1	1	1
102	1	1	0	1	1	1	0
103	0	0	1	1	1	1	0
104	0	0	0	1	0	0	0
105	0	0	0	1	1	1	1
106	0	0	0	1	1	1	1
107	0	0	0	1	1	1	0
108	0	0	0	1	1	0	1
109	1	0	0	1	1	1	1
110	0	0	1	1	1	0	0
111	0	0	0	1	1	1	1
112	0	0	0	1	1	1	1
113	0	0	0	1	1	0	0
114	1	1	1	1	1	0	0
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	acetate	aconitat	b.alanin	d.alanin	l.alanin	y.a.buty	l.arabin
94	1	0	0	1	1	0	0
95	1	0	0	1	1	1	0
96	1	1	1	1	1	1	1
97	1	0	1	1	1	1	1
98	1	0	0	0	1	0	0
99	0	0	0	1	0	1	0
100	1	0	1	1	1	0	0
101	1	0	0	1	1	0	0
102	1	0	0	0	0	0	1
103	1	0	1	1	1	0	1
104	0	0	1	1	1	0	0
105	1	0	1	1	1	0	1
106	1	0	1	1	1	0	1
107	1	1	1	1	1	1	1
108	1	0	1	1	1	0	1
109	1	1	1	1	1	1	1
110	0	0	1	1	1	1	1
111	1	0	0	1	1	0	1
112	1	0	0	0	0	1	1
113	1	0	1	1	1	0	1
114	1	0	0	1	1	1	0
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	l.aspart	caprate	caproate	caprylat	cellobio	citrate	citrulli
94	1	1	1	1	1	0	1
95	1	1	1	1	0	1	1
96	1	1	1	1	0	1	1
97	1	1	1	1	1	1	1
98	1	1	1	1	1	0	1
99	0	0	0	0	0	0	0
100	1	0	1	1	1	0	0
101	1	1	1	1	1	1	1
102	1	0	0	0	1	1	0
103	1	1	1	1	1	1	1
104	1	0	0	0	0	0	1
105	1	1	1	1	0	1	1
106	1	1	0	0	0	1	1
107	1	1	1	1	1	1	1
108	1	1	0	1	1	1	1
109	1	1	1	1	0	1	1
110	1	1	0	0	1	1	1
111	1	1	0	1	1	1	1
112	1	1	1	1	1	1	1
113	1	1	1	1	1	1	1
114	1	1	0	1	0	1	0
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	ethanol	d.galact	d.gal.u	d.glucon	l.glutam	glutarat	d.glucur
94	0	0	0	0	1	0	0
95	0	0	0	0	1	1	0
96	1	0	0	1	1	0	0
97	1	0	1	1	1	1	1
98	0	0	0	0	1	0	0
99	0	0	0	1	0	0	0
100	0	0	0	0	1	0	0
101	0	1	0	1	1	0	0
102	0	0	0	0	1	0	1
103	1	1	1	1	1	1	1
104	1	0	0	0	1	0	0
105	1	0	0	1	1	0	0
106	1	1	0	1	1	0	0
107	1	1	1	1	1	0	1
108	1	0	0	1	1	0	1
109	1	1	1	1	1	1	1
110	1	1	1	1	1	0	1
111	0	1	0	1	1	0	1
112	0	1	0	1	1	0	1
113	0	1	0	1	1	0	1
114	0	0	0	0	1	1	0
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	dl.glyce	glycine	heptanoa	l.histid	p.hydrox	b.hydrox	inositol
94	0	1	1	0	0	0	0
95	0	0	1	1	0	1	0
96	1	1	1	1	0	1	0
97	1	1	1	1	0	1	0
98	0	1	1	0	0	1	0
99	0	0	0	0	0	1	0
100	1	1	1	1	0	1	1
101	1	0	1	1	0	0	0
102	0	0	0	0	0	0	0
103	1	0	1	1	0	1	0
104	1	1	0	1	0	0	0
105	1	1	1	0	0	0	0
106	1	1	1	1	0	0	0
107	1	1	1	1	1	1	0
108	1	1	1	0	0	1	0
109	1	1	1	1	1	1	0
110	1	1	1	1	0	0	1
111	1	1	1	1	0	0	1
112	0	0	1	0	0	0	1
113	1	1	1	1	0	1	1
114	1	0	1	1	0	1	0
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	isobutyr	a.ketogl	dl.lacta	lactose	l.leucin	dl.malat	d.mannit
94	0	0	1	1	0	1	1
95	1	0	1	1	1	1	0
96	1	0	1	1	1	1	1
97	1	0	1	1	1	1	1
98	1	0	1	1	0	1	1
99	0	0	0	1	0	0	0
100	0	0	1	1	0	1	1
101	0	0	1	1	0	1	0
102	0	0	1	1	0	1	1
103	1	0	1	1	1	1	1
104	0	0	0	0	1	1	0
105	0	0	1	0	0	1	1
106	0	0	1	0	0	1	1
107	0	0	1	0	0	1	1
108	0	0	1	0	0	1	1
109	0	0	1	0	1	1	1
110	0	0	1	1	1	1	1
111	0	0	1	0	0	1	1
112	0	0	1	0	0	1	1
113	0	0	1	1	0	1	1
114	1	0	1	0	1	1	0
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	d.mannos	melibios	l.ornith	pelargon	l.prolin	propanol	propiona
94	1	0	1	0	1	0	1
95	1	0	1	1	1	0	1
96	1	1	1	1	1	1	1
97	1	1	1	1	1	1	1
98	1	0	1	1	1	0	1
99	0	0	0	0	0	0	0
100	1	1	1	0	1	0	1
101	1	0	1	0	1	0	1
102	1	0	0	0	1	0	1
103	1	0	1	1	1	1	1
104	0	0	1	0	1	1	1
105	1	0	1	0	1	1	1
106	1	0	1	0	1	1	1
107	1	0	1	1	1	1	1
108	1	0	1	0	1	1	1
109	1	0	1	1	1	1	1
110	1	0	1	0	1	1	1
111	1	0	0	1	1	1	1
112	1	0	0	0	1	0	1
113	1	1	1	0	1	0	1
114	0	0	0	1	1	0	1
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	putresci	pyruvate	quinate	l.rhamno	d.ribose	salicin	l.serine
94	0	1	0	0	1	0	1
95	1	1	0	0	1	0	0
96	1	1	0	0	1	0	1
97	1	1	0	0	1	0	1
98	0	1	0	0	1	0	1
99	1	0	0	0	0	0	0
100	0	1	0	0	1	0	1
101	0	1	0	0	1	0	1
102	0	1	0	0	1	1	1
103	1	1	0	1	1	1	1
104	0	0	0	0	1	0	1
105	0	1	1	0	0	1	1
106	0	1	0	0	1	1	1
107	0	1	1	0	1	1	1
108	1	1	0	1	0	1	1
109	1	1	1	1	1	1	1
110	1	1	0	1	1	1	1
111	0	1	0	0	1	1	1
112	0	1	0	0	1	1	1
113	1	1	1	0	1	1	1
114	1	1	0	0	0	0	1
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	d.sorbit	sucrose	trehalos	l.tyrosi	valerate	d.xylose	amylase
94	0	0	1	1	0	0	1
95	0	0	1	1	1	0	0
96	1	1	1	1	1	0	0
97	1	0	1	1	1	1	1
98	0	0	1	1	1	0	1
99	0	1	0	0	0	0	1
100	1	0	1	1	0	0	1
101	0	0	1	1	0	0	1
102	0	1	1	0	0	1	1
103	1	1	1	1	1	1	1
104	0	1	0	1	0	1	0
105	1	0	1	1	0	1	0
106	0	0	1	1	0	1	1
107	0	1	1	1	0	1	1
108	0	1	1	1	0	1	1
109	0	1	1	1	1	1	1
110	1	1	1	1	0	1	1
111	0	1	1	0	0	1	1
112	0	1	1	0	0	1	1
113	1	1	1	1	0	1	0
114	0	0	0	1	1	0	1
115	.	.	.	.	.	.	.
116	.	.	.	.	.	.	.
117	.	.	.	.	.	.	.
118	.	.	.	.	.	.	.
119	.	.	.	.	.	.	.
120	.	.	.	.	.	.	.
121	.	.	.	.	.	.	.

	gelatin	lipase	alginase
94	1	1	0
95	0	1	0
96	0	0	0
97	1	1	0
98	1	1	0
99	1	1	0
100	1	0	0
101	1	1	0
102	1	1	0
103	0	0	0
104	1	0	0
105	0	0	0
106	1	1	0
107	1	1	0
108	1	1	0
109	1	1	0
110	1	1	0
111	1	1	0
112	0	0	0
113	0	1	0
114	1	1	0
115	.	.	.
116	.	.	.
117	.	.	.
118	.	.	.
119	.	.	.
120	.	.	.
121	.	.	.

		strain	a2	a3	a4	a5	a6	a7	a8	a9	a1	a11	a12	b1	b2
1	1		0	0	0	1	1	0	0	0	0	0	0	0	0
2	2		0	1	1	1	1	0	0	0	0	0	1	0	0
3	3		0	0	0	1	1	0	0	0	0	0	0	0	0
4	4		0	1	1	1	1	0	0	0	0	0	1	0	1
5	5		0	0	0	1	1	0	0	0	0	0	0	0	0
6	6		1	1	1	1	1	0	0	0	0	0	1	0	0
7	7		1	1	1	1	1	0	0	0	0	0	1	0	0
8	8		0	1	1	1	1	0	0	0	0	0	1	0	0
9	9		0	0	0	1	1	0	0	0	0	0	0	0	0
10	10		0	0	0	1	1	0	0	0	0	0	0	0	0
11	11		0	0	0	1	1	0	0	0	0	0	0	0	0
12	12		0	0	0	1	1	0	0	0	0	0	0	0	0
13	13		1	1	0	1	1	0	0	0	0	0	1	0	1
14	14		1	1	1	1	1	0	0	0	0	0	1	0	0
15	15		1	0	0	1	1	0	0	0	0	0	1	0	1
16	16		0	0	0	1	1	0	1	0	1	1	0	0	1
17	17		0	0	0	1	1	0	0	0	0	0	0	1	0
18	18		0	0	0	1	1	0	0	0	0	0	0	0	0
19	19		0	0	0	1	1	0	0	0	0	0	0	0	0
20	20		0	0	0	1	1	0	0	0	0	0	1	0	1
21	21		0	1	1	1	1	0	1	0	0	0	1	0	1
22	22		0	1	1	1	1	0	1	0	0	0	1	0	1
23	23		0	1	1	1	1	0	0	0	0	0	0	0	0
24	24		0	1	1	1	1	0	1	0	0	0	1	0	1
25	25		0	0	0	1	1	0	0	0	0	0	0	0	0
26	26		1	1	1	1	0	0	1	0	0	0	1	0	1
27	27		0	1	1	1	1	0	1	0	0	0	1	0	1
28	28		0	1	1	1	1	0	1	0	0	0	1	0	1
29	29		0	1	1	1	1	0	1	0	0	0	1	0	1
30	30		0	1	1	1	1	0	1	0	1	0	1	0	1
31	31		0	1	1	1	0	0	1	0	0	0	1	0	1

	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	c1	c2	c3	c4
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	1	0	1	0	1	1	1	1	0	1	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	1	0	1	0	1	1	1	1	1	1	0	1	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	1	0	1	0	1	0	1	1	0	1	0	0	0
7	0	1	0	1	0	1	0	1	1	0	1	0	0	0
8	0	1	1	1	0	1	1	1	1	0	1	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	1	0	1	0	0	0	1	1	0	1	0	1	0
14	0	1	0	1	0	1	1	1	1	0	1	0	0	0
15	0	1	0	1	0	1	0	1	1	0	1	0	1	0
16	0	0	0	1	0	0	0	1	1	1	0	0	1	0
17	0	0	0	0	0	0	0	0	0	0	1	1	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	1	0	1	0	0	0	1	1	0	1	0	1	0
21	0	1	0	1	0	0	0	1	1	1	0	0	1	0
22	0	1	0	1	0	0	0	1	1	1	0	0	1	0
23	0	0	0	1	0	0	0	1	1	0	0	0	0	0
24	0	1	0	1	0	0	0	1	1	1	0	0	1	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	1	0	1	0	0	0	1	1	1	0	0	1	0
27	0	1	0	1	0	0	0	1	1	1	0	0	1	0
28	0	1	0	1	0	0	0	1	1	1	0	0	1	0
29	0	1	0	1	0	0	0	1	1	1	0	0	1	0
30	0	1	0	1	0	0	0	1	1	1	0	0	1	0
31	0	1	0	1	0	0	0	1	1	1	0	0	1	0

	c5	c6	c7	c8	c9	c10	c11	c12	d1	d2	d3	d4	d5	d6
1	0	0	0	0	0	0	1	1	1	1	1	0	0	0
2	0	0	1	0	0	0	1	1	0	0	1	0	0	0
3	0	0	0	0	0	0	1	1	1	1	0	0	0	0
4	0	0	1	1	0	0	1	1	1	1	1	0	0	0
5	0	0	0	0	0	0	1	1	1	1	0	0	0	0
6	0	0	1	1	0	0	1	1	1	1	0	0	0	0
7	0	0	1	1	0	0	1	1	1	1	0	0	0	0
8	0	0	1	0	0	0	1	1	1	1	1	0	0	0
9	0	0	0	0	0	0	1	1	1	1	1	0	0	0
10	0	0	0	0	0	0	1	1	1	1	0	0	0	0
11	0	0	0	0	0	0	1	1	1	0	0	0	0	0
12	0	0	0	0	0	0	1	1	1	1	1	0	0	0
13	0	0	1	1	0	0	1	1	1	0	0	0	0	0
14	0	0	1	0	0	0	1	1	1	1	1	0	0	0
15	0	0	1	1	0	0	1	1	1	1	1	0	0	0
16	0	1	1	1	1	1	1	1	1	1	0	1	0	0
17	0	0	0	0	0	0	1	1	1	1	1	0	0	0
18	0	0	0	0	0	0	1	1	1	1	1	0	0	0
19	0	0	0	0	0	0	1	0	1	1	1	0	0	0
20	0	0	1	1	0	0	1	1	1	1	0	0	0	0
21	0	0	0	1	0	0	1	0	1	1	1	0	0	1
22	0	0	0	1	0	0	0	0	1	0	0	0	0	0
23	0	0	0	0	0	0	1	0	0	1	1	1	0	1
24	0	0	0	1	0	0	1	0	0	0	0	0	0	0
25	0	0	0	0	0	0	1	1	1	1	1	0	0	0
26	0	0	0	1	0	0	0	0	0	0	0	0	0	0
27	0	0	0	1	0	0	1	0	0	0	0	0	0	0
28	0	0	0	1	0	0	1	0	0	0	0	0	0	0
29	0	0	0	1	0	0	1	0	1	1	1	0	0	1
30	0	0	0	1	0	0	1	1	1	1	1	0	0	1
31	0	0	0	1	0	0	0	0	0	0	0	0	0	0

	d7	d8	d9	d10	d11	d12	e1	e2	e3	e4	e5	e6	e7	e8
1	0	0	0	0	1	0	0	0	0	1	1	1	0	1
2	1	0	0	0	0	0	0	1	0	0	0	0	0	0
3	0	0	0	0	1	0	0	0	0	1	1	1	0	1
4	1	0	0	0	1	0	0	0	1	0	0	0	0	1
5	0	0	0	0	1	0	0	0	0	1	1	1	0	1
6	1	0	0	0	1	0	0	1	0	0	0	0	0	1
7	1	0	0	0	1	0	0	0	0	1	0	0	0	1
8	1	0	0	0	0	0	0	0	0	0	0	0	0	1
9	0	0	0	0	1	0	0	0	0	1	1	1	0	1
10	0	0	0	0	1	0	0	0	0	1	1	1	0	1
11	0	0	0	0	1	0	0	0	0	0	0	1	0	1
12	0	0	0	0	1	0	0	0	0	1	1	1	0	1
13	1	0	0	0	0	0	0	0	0	0	0	0	0	1
14	1	0	0	0	0	0	0	0	0	0	0	0	0	1
15	1	0	0	0	1	0	0	0	0	0	0	0	0	1
16	1	1	0	1	1	0	0	1	1	1	1	1	1	1
17	0	0	0	0	1	0	1	0	0	1	1	1	0	1
18	0	0	0	0	1	0	0	1	0	1	1	1	0	1
19	0	0	0	0	1	0	0	1	0	1	1	1	0	1
20	1	0	0	0	1	0	0	0	0	0	0	0	0	0
21	1	0	1	0	1	0	0	0	0	1	0	1	1	1
22	1	0	0	0	0	0	0	0	0	1	0	1	0	0
23	1	0	1	0	1	0	0	0	0	1	0	1	1	0
24	1	0	0	0	0	0	0	1	0	1	0	1	0	0
25	0	0	0	0	1	0	0	0	0	1	1	1	0	1
26	1	0	0	0	0	0	0	0	0	1	0	1	0	0
27	1	0	0	0	0	0	0	0	0	1	0	1	0	0
28	1	0	1	0	0	0	0	0	0	1	0	1	0	0
29	1	0	1	0	1	0	0	0	0	1	0	1	1	0
30	1	0	1	0	1	0	0	0	0	1	0	1	1	1
31	1	0	0	0	0	0	0	0	0	0	0	1	0	0

	e9	e10	e11	e12	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
1	0	0	0	1	1	1	0	1	1	1	1	1	1	1
2	0	0	0	1	1	1	0	0	0	1	1	1	1	1
3	0	0	0	1	1	1	0	0	1	1	1	1	1	1
4	0	0	0	1	0	1	0	1	0	1	1	1	1	1
5	0	0	0	0	0	1	0	0	1	1	1	1	1	1
6	0	0	0	1	1	1	0	0	0	1	1	1	1	1
7	0	0	0	1	1	1	0	1	0	1	1	1	1	1
8	0	0	0	1	0	1	0	0	0	1	1	1	0	1
9	0	0	0	0	0	1	0	1	1	1	1	1	1	1
10	0	0	0	0	1	1	0	1	1	1	1	1	1	1
11	0	0	0	1	0	0	0	0	1	1	1	0	1	1
12	0	0	0	1	1	1	0	0	1	1	1	1	1	1
13	0	0	0	1	0	1	0	1	0	1	1	1	1	1
14	0	0	0	1	1	1	0	1	0	1	1	1	1	1
15	0	0	0	1	0	1	0	0	0	1	1	1	1	1
16	1	1	1	1	1	1	0	0	1	1	0	1	1	1
17	0	0	0	1	1	1	0	1	1	1	1	0	1	1
18	0	0	0	1	1	1	0	0	1	1	1	1	1	1
19	0	0	0	0	1	1	0	0	1	1	1	0	1	1
20	0	0	0	1	1	1	0	0	0	1	1	1	1	1
21	0	1	0	1	1	0	0	0	1	1	1	1	1	1
22	0	0	0	1	0	0	0	0	1	0	1	1	1	1
23	0	1	0	1	1	0	0	0	1	1	0	1	0	1
24	0	0	0	1	0	0	0	0	1	1	1	1	1	1
25	0	0	0	1	1	1	0	0	1	1	1	1	1	1
26	0	0	0	1	0	0	0	0	1	1	1	1	1	1
27	0	0	0	1	0	0	0	0	1	1	1	1	1	1
28	0	0	0	1	1	0	1	0	1	1	1	1	1	1
29	0	1	0	1	1	0	1	0	1	1	1	1	1	1
30	0	1	0	1	1	1	0	0	1	1	1	1	1	1
31	0	0	0	1	0	0	0	0	1	0	0	1	1	1

	f11	f12	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11	g12
1	0	1	1	1	1	0	1	1	1	0	1	0	0	1
2	1	1	0	0	1	0	0	1	0	0	1	1	0	0
3	0	1	0	1	1	0	1	1	0	0	0	0	0	1
4	1	1	0	0	1	0	0	1	0	0	1	1	0	0
5	0	0	0	1	1	0	1	1	0	0	1	0	0	1
6	1	1	0	0	1	0	0	1	0	0	1	1	0	0
7	1	1	0	0	1	0	0	1	1	0	1	1	0	0
8	1	1	0	0	1	0	0	1	0	0	1	1	0	0
9	0	1	1	1	1	0	1	1	0	0	1	0	0	1
10	0	1	0	1	1	0	1	1	1	0	1	0	0	1
11	0	1	0	0	1	0	1	1	0	0	1	0	0	1
12	0	1	1	1	1	0	1	1	0	0	1	0	0	1
13	1	1	0	0	1	0	0	1	0	0	1	1	0	0
14	1	1	0	0	1	0	0	1	0	0	1	1	0	0
15	1	1	0	0	1	0	0	1	0	0	1	1	0	0
16	0	0	0	0	1	0	0	1	0	0	1	1	0	0
17	0	1	1	1	1	0	1	1	1	0	0	0	0	1
18	0	1	1	1	1	0	1	1	0	0	1	0	0	1
19	0	1	1	1	1	0	0	1	0	0	1	0	0	1
20	1	1	0	0	1	0	0	1	0	0	1	1	0	0
21	1	1	1	0	1	0	0	1	0	0	1	1	0	1
22	1	1	0	0	0	1	0	1	0	0	1	1	0	0
23	0	0	0	0	1	0	1	1	0	0	0	0	0	0
24	1	1	0	0	0	0	0	1	0	0	1	1	0	0
25	0	1	0	1	1	0	1	1	0	0	1	0	0	1
26	1	0	0	0	0	0	0	1	0	0	1	1	0	0
27	1	1	0	0	0	0	0	1	0	0	1	1	0	0
28	1	1	0	0	0	0	0	1	0	0	1	1	0	0
29	1	1	0	0	1	0	0	1	0	0	1	1	0	1
30	1	1	1	1	1	0	1	1	0	0	1	1	0	1
31	1	1	0	0	0	0	0	1	0	0	0	1	0	0

	h1	h2	h3	h4	h5	h6	h7	h8	h9	h10	h11	h12
1	1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	1	0	0	0	0	1	0	0	0	0
3	1	0	0	0	0	0	0	0	0	0	0	0
4	0	1	1	0	0	0	0	1	0	0	0	0
5	1	0	0	0	0	0	0	1	0	0	0	0
6	0	0	1	0	0	0	0	1	0	0	0	0
7	0	0	1	0	0	0	0	0	0	0	0	0
8	0	0	1	0	0	0	0	0	0	0	0	0
9	1	0	0	0	0	0	0	1	0	0	0	0
10	1	0	0	0	0	0	0	1	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	1	0	0	0	1	0	0	0	0	0	0	0
13	0	0	1	0	0	0	0	1	0	0	0	0
14	0	1	1	0	0	0	0	1	0	0	0	0
15	0	0	1	0	0	0	0	0	0	0	0	0
16	0	0	0	1	0	1	1	1	1	0	0	0
17	1	0	0	0	0	0	0	1	0	0	0	0
18	1	0	0	0	0	0	0	1	0	0	0	0
19	1	0	0	0	0	0	0	1	0	0	0	0
20	0	0	1	0	0	0	0	0	0	0	0	0
21	1	1	1	1	0	0	0	0	1	0	1	1
22	0	1	1	1	0	0	0	0	1	0	1	1
23	0	1	0	0	0	0	0	0	1	0	0	0
24	0	1	1	1	0	0	0	0	1	0	1	1
25	1	0	0	0	0	0	0	0	0	0	0	0
26	0	1	1	1	0	0	0	0	1	1	1	1
27	0	1	1	1	0	0	0	0	1	0	1	1
28	0	1	1	1	0	0	0	0	1	0	1	1
29	0	1	1	1	0	0	0	0	1	0	1	1
30	1	1	1	1	0	0	0	0	1	0	1	1
31	0	1	1	1	0	0	0	0	1	0	1	1

	strain	a2	a3	a4	a5	a6	a7	a8	a9	a1	a11	a12	b1	b2
32	32	0	1	1	1	1	0	0	0	0	0	0	0	1
33	33	0	0	0	1	1	0	0	0	0	0	0	0	0
34	34	0	0	0	1	0	0	0	0	0	0	1	0	0
35	35	0	1	1	1	1	0	1	0	0	0	1	0	1
36	36	0	1	1	1	1	0	1	0	0	0	1	0	1
37	37	0	0	0	1	1	0	0	0	0	0	0	0	0
38	38	0	1	1	1	1	0	0	0	0	0	0	0	0
39	39	0	1	1	1	1	0	1	0	0	0	0	0	0
40	40	0	0	0	1	1	0	0	0	0	0	0	0	0
41	41	0	0	0	1	1	0	0	0	0	0	0	0	0
42	42	0	1	0	1	1	0	1	0	0	0	0	0	1
43	43	0	0	0	1	1	0	0	0	0	0	0	0	0
44	44	0	1	0	1	1	0	0	0	0	0	0	0	0
45	45	1	1	1	1	1	0	1	0	0	0	1	0	0
46	46	1	1	1	1	1	0	1	0	0	0	1	0	1
47	47	1	1	1	1	1	0	0	0	0	0	1	0	1
48	48	0	1	0	1	1	0	0	0	0	0	0	0	1
49	49	1	0	0	1	1	0	0	0	0	0	0	0	0
50	50	0	1	1	1	1	0	0	0	0	0	0	0	0
51	51	0	1	1	1	1	0	1	0	0	0	0	0	1
52	52	0	0	0	1	1	0	0	0	0	0	0	0	0
53	53	0	0	0	1	1	0	0	0	0	0	0	0	0
54	54	0	1	1	1	1	0	1	0	0	0	1	0	1
55	55	0	0	0	1	1	0	0	0	0	0	0	0	0
56	56	0	0	0	0	0	0	0	0	0	0	0	0	1
57	57	0	1	1	1	1	1	1	0	0	0	0	0	1
58	58	0	1	1	1	1	0	1	0	0	0	1	0	1
59	59	0	1	1	1	1	0	1	0	0	0	1	0	1
60	60	1	0	0	1	1	0	0	0	0	0	0	0	0
61	61	0	1	1	1	1	0	0	0	0	0	0	0	1
62	62	0	1	1	1	1	1	1	0	1	0	1	0	1

	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	c1	c2	c3	c4
32	0	0	0	1	0	0	0	1	1	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	1	0	1	0	0	0	1	1	1	0	0	1	0
36	0	1	0	1	0	0	0	1	1	1	0	0	1	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	1	0	0	0	1	1	0	0	0	0	0
39	0	0	0	1	0	0	0	1	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	1	1	1	0	1	0	1	0	0	0	1	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	1	0	0	0	1	0	0	0	0	0	0
45	0	0	0	1	0	0	0	1	1	0	0	0	0	0
46	0	0	0	1	0	0	0	1	1	0	0	0	0	0
47	0	1	0	1	0	1	0	1	1	0	1	0	0	0
48	0	1	0	1	1	0	0	1	1	1	0	0	1	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	1	0	0	0	1	1	0	0	0	0	0
51	0	0	0	1	0	0	0	1	1	0	0	0	1	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	1	0	1	0	0	0	1	1	1	0	0	1	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	1	0	0	0	0
57	0	1	0	1	0	0	0	1	1	1	0	1	1	0
58	0	1	0	1	0	0	0	1	1	1	0	0	1	0
59	0	0	0	1	0	0	0	1	1	1	0	0	1	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	0	1	0	1	1	0	1	1	1	0	0	0	1	0
62	0	1	0	1	0	0	0	1	1	1	0	1	1	0

	c5	c6	c7	c8	c9	c10	c11	c12	d1	d2	d3	d4	d5	d6
32	0	0	0	0	0	0	1	0	1	1	1	1	0	1
33	0	0	0	0	0	0	1	1	1	1	0	0	0	0
34	0	0	0	0	0	0	0	0	1	0	0	0	0	1
35	0	0	0	1	0	0	1	0	0	0	0	0	0	0
36	0	0	0	1	0	0	1	0	0	0	0	0	0	0
37	0	0	0	0	0	0	1	1	1	1	1	0	0	0
38	0	0	0	0	0	0	1	0	0	1	1	1	0	1
39	0	0	1	0	0	0	1	0	0	0	0	0	0	0
40	0	0	0	0	0	0	1	1	1	1	0	0	0	0
41	0	0	0	0	0	0	1	1	1	1	0	0	0	0
42	0	0	0	0	0	0	1	1	1	1	0	0	0	0
43	0	0	0	0	0	0	1	1	1	1	0	1	0	0
44	0	0	0	0	0	0	1	1	0	1	1	0	0	1
45	0	0	1	0	0	0	1	0	1	0	0	0	0	0
46	0	0	1	0	0	0	1	1	1	0	0	0	0	0
47	0	0	1	0	0	0	1	1	1	1	1	0	0	0
48	1	0	1	1	1	0	1	0	0	1	1	0	0	0
49	0	0	0	0	0	0	1	1	1	1	1	0	0	0
50	0	0	0	0	0	0	1	1	1	1	1	1	0	1
51	0	0	1	0	0	0	1	0	1	1	1	0	0	1
52	0	0	0	0	0	0	1	1	1	1	0	0	0	0
53	0	0	0	0	0	0	1	1	1	1	1	0	0	0
54	0	0	1	1	0	0	1	1	1	1	0	0	0	0
55	0	0	0	0	0	0	1	1	1	1	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	1	1	0	0	1	1	1	0	0	0	0	0
58	0	1	0	1	0	0	1	0	1	0	0	0	0	0
59	0	1	0	1	0	0	1	0	1	0	0	0	0	0
60	0	0	0	0	0	0	1	1	1	1	0	0	0	0
61	0	0	1	1	1	0	1	1	0	1	1	1	1	0
62	0	1	1	1	0	0	1	1	1	1	1	1	0	1

	d7	d8	d9	d10	d11	d12	e1	e2	e3	e4	e5	e6	e7	e8
32	1	0	1	1	1	0	0	0	0	1	0	1	1	1
33	0	0	0	0	1	0	0	0	0	1	1	1	0	1
34	0	0	0	0	0	0	0	0	0	0	0	0	0	1
35	1	0	0	0	0	0	0	0	0	1	0	1	0	0
36	1	0	0	0	0	0	0	0	0	1	0	1	0	0
37	0	0	0	0	1	0	0	0	0	1	1	1	0	1
38	1	0	1	1	1	0	0	0	0	1	0	1	1	0
39	0	0	0	0	0	0	0	0	0	0	0	1	0	0
40	0	0	0	0	1	0	0	0	0	1	1	1	0	1
41	0	0	0	0	1	0	0	0	0	1	1	1	0	1
42	0	0	0	0	1	0	0	0	0	1	1	1	0	1
43	0	0	0	0	1	0	0	0	0	1	1	1	1	1
44	1	0	1	0	1	0	0	0	0	1	1	1	1	1
45	0	0	0	0	0	0	0	0	0	0	1	1	0	1
46	1	0	0	1	1	0	0	0	1	1	1	1	0	1
47	1	0	0	0	0	0	0	0	1	0	1	0	0	1
48	1	0	1	0	1	1	0	0	0	1	0	1	0	0
49	0	0	0	0	1	0	0	1	0	1	1	1	0	1
50	1	0	1	1	1	0	0	1	0	1	1	1	1	1
51	0	0	1	0	1	0	0	0	0	1	0	1	1	1
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53	0	0	0	0	1	0	0	0	0	1	0	1	0	1
54	1	0	1	0	1	0	0	0	0	1	0	1	1	0
55	0	0	0	0	1	0	0	0	0	1	1	1	0	1
56	0	0	0	0	0	0	0	1	0	0	0	0	0	0
57	1	0	0	0	1	0	0	0	0	0	0	1	0	1
58	1	0	0	0	0	0	0	0	0	1	0	1	0	0
59	1	0	0	0	0	0	0	1	0	0	0	1	0	0
60	0	0	0	0	1	0	0	0	0	1	1	1	0	1
61	1	0	0	0	1	0	0	0	0	1	0	1	0	0
62	1	0	1	0	1	0	0	0	0	1	0	1	1	0

	e9	e10	e11	e12	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
32	0	1	0	1	1	0	0	1	0	1	0	1	0	1
33	0	0	0	1	1	1	0	1	1	1	1	1	1	1
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	1	0	0	0	0	1	1	1	1	1	1
36	0	0	0	1	0	0	0	0	1	1	1	1	1	1
37	0	0	0	1	1	1	0	1	1	1	1	1	1	1
38	0	1	0	1	1	0	0	1	0	1	0	1	0	1
39	0	0	0	0	0	0	0	0	0	1	0	0	0	0
40	0	0	0	1	1	1	0	0	1	1	0	1	1	1
41	0	0	0	1	1	1	0	1	1	1	1	1	1	1
42	0	0	0	0	1	1	0	1	1	1	1	1	1	1
43	0	0	0	1	1	1	0	0	1	1	0	1	1	1
44	0	1	0	1	1	1	0	0	1	1	0	1	1	1
45	0	0	0	0	0	0	0	0	0	1	1	1	1	1
46	0	0	0	1	1	1	0	1	0	1	1	1	1	1
47	0	0	0	1	0	1	0	0	0	1	1	1	1	1
48	0	0	0	1	1	1	0	0	1	1	1	1	1	1
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53	0	0	0	1	0	1	0	1	1	1	0	1	1	1
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55	0	0	0	1	1	1	0	1	1	1	1	1	1	1
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	1	1	0	0	0	1	1	1	1	1	1
58	0	0	0	1	0	0	0	0	1	1	1	1	1	1
59	0	0	0	1	0	0	0	0	1	1	1	1	1	1
60	0	0	0	1	1	1	0	0	1	1	1	1	1	1
61	0	0	0	1	1	1	0	0	1	1	1	1	1	1
62	0	1	0	1	1	0	1	0	1	1	1	1	1	1

	f11	f12	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11	g12
32	0	0	0	0	1	0	0	1	0	0	0	0	0	0
33	0	1	0	1	1	0	1	1	0	0	1	0	0	1
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	1	1	0	0	0	0	0	1	0	0	1	1	0	0
36	1	1	0	0	0	0	0	1	0	0	1	1	0	0
37	0	1	1	1	1	0	1	1	0	0	1	0	0	1
38	0	0	0	0	1	0	0	1	0	0	1	0	0	0
39	0	0	0	0	1	0	0	0	0	0	0	0	0	0
40	0	1	0	1	1	0	1	1	0	0	1	0	0	1
41	0	1	0	1	1	0	1	1	0	0	1	0	0	1
42	0	1	1	1	1	0	1	1	0	0	1	0	0	1
43	0	1	1	1	1	0	1	1	0	0	1	0	0	1
44	0	0	0	0	1	0	1	1	0	0	1	0	0	1
45	1	1	0	0	1	0	0	0	0	0	1	1	0	0
46	1	1	0	0	1	0	0	0	0	1	1	1	0	0
47	1	1	0	0	1	0	0	1	0	0	1	1	0	0
48	0	1	0	0	0	0	0	1	1	0	1	0	0	1
49	0	1	1	1	1	0	1	1	1	1	1	0	0	1
50	0	1	1	1	1	0	1	1	0	1	1	0	0	1
51	0	1	0	0	1	0	1	1	0	0	1	0	0	0
52	0	1	1	1	1	0	1	1	0	0	1	0	0	1
53	0	1	0	1	1	0	1	1	0	0	1	0	0	1
54	1	1	0	0	1	0	0	1	0	0	1	1	0	1
55	0	1	1	1	1	0	1	1	0	0	1	0	0	1
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	1	1	1	0	0	0	1	1	0	0	1	0	0	1
58	1	1	0	0	0	0	0	1	0	0	1	1	0	0
59	1	1	0	0	0	0	0	0	0	0	1	1	1	0
60	0	1	1	1	1	0	1	1	0	0	1	0	0	1
61	0	1	0	0	0	0	0	1	1	0	1	0	1	1
62	1	1	1	0	0	0	0	1	0	1	1	1	0	0

	h1	h2	h3	h4	h5	h6	h7	h8	h9	h10	h11	h12
32	0	1	0	0	0	0	0	0	1	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	1	0	0	0	0	0	0	0
35	0	1	1	1	0	0	0	0	1	0	1	1
36	0	1	1	1	0	0	0	0	1	0	1	1
37	1	0	0	0	0	0	0	0	0	0	0	0
38	0	1	0	0	0	0	0	0	1	0	0	0
39	0	1	1	1	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	1	0	0	0	0	0	0	0	0	0	0	0
42	1	1	0	1	0	0	0	0	1	0	0	0
43	1	0	0	0	0	0	0	0	0	0	0	0
44	0	1	0	0	0	0	0	0	1	0	0	0
45	0	1	1	1	0	0	0	0	0	0	0	0
46	0	1	1	1	0	0	0	0	0	0	0	0
47	0	1	1	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	1	1	0	0
49	1	1	0	0	0	1	0	1	0	0	1	0
50	1	1	0	0	0	0	0	0	1	0	0	0
51	0	1	0	0	0	0	0	0	1	0	0	0
52	1	0	0	0	0	0	0	0	0	0	0	0
53	0	1	0	0	0	0	0	0	0	0	0	0
54	0	1	1	1	0	0	0	0	1	0	1	1
55	1	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	1	1	0	1	0	0	0	0	1	1	1	1
58	1	1	1	1	0	0	0	1	1	0	1	1
59	0	1	0	1	1	0	0	0	1	0	1	1
60	1	0	0	0	0	0	0	0	0	0	0	1
61	0	1	0	0	0	1	0	0	1	0	0	0
62	1	1	1	1	0	0	0	0	1	1	1	1

	strain	a2	a3	a4	a5	a6	a7	a8	a9	a1	a11	a12	b1	b2
63	63	0	1	1	1	1	0	1	0	0	0	1	0	1
64	64	0	1	1	1	1	0	1	0	1	0	1	0	1
65	65	0	1	1	1	1	1	1	0	0	0	1	0	1
66	66	0	1	1	1	0	0	1	0	0	0	0	0	0
67	67	1	1	1	1	1	0	1	0	0	0	1	0	0
68	68	0	1	1	1	1	0	1	0	0	0	0	0	0
69	69	0	1	1	1	1	0	1	0	0	0	1	0	0
70	70	0	1	1	1	1	0	1	0	0	0	0	0	0
71	71	0	1	1	1	1	0	1	0	0	0	1	0	0
72	72	0	0	0	1	1	0	0	0	0	0	0	0	0
73	73	0	0	0	1	1	0	0	0	0	0	0	0	0
74	74	0	1	1	1	1	0	0	0	0	0	0	0	0
75	75	0	1	1	1	1	0	0	0	0	1	0	0	1
76	76	1	1	1	1	0	0	1	0	0	0	1	0	1
77	77	0	1	1	1	1	0	0	0	0	1	0	0	0
78	78	0	1	1	1	1	0	1	0	0	0	1	0	1
79	79	1	1	1	1	1	0	1	0	1	0	1	0	1
80	80	1	1	1	1	1	0	1	0	0	0	1	0	1
81	V. aestuarianus *	1	1	1	1	1	1	1	0	0	0	1	0	1
82	V. alginolyticus *	1	1	1	1	1	0	1	0	0	0	0	0	1
83	L. anguillarum *	1	1	1	1	1	1	1	0	0	0	1	0	1
84	V. campbellii *	1	1	1	1	1	0	1	0	1	0	1	0	1
85	V. carchariae *	1	1	1	1	1	0	1	0	0	0	1	0	1
86	V. cincinnat. *	0	1	1	1	1	0	1	0	1	0	1	0	1
87	V. costicola *	0	1	1	0	0	0	1	0	0	0	0	0	1
88	L. damsela *	0	1	1	1	1	1	1	0	0	0	1	0	1
89	V. diazotrophicus *	1	1	1	1	1	0	1	0	1	0	1	0	1
90	V. fisheri *	0	0	0	0	0	0	1	0	0	0	0	0	1
91	V. fluvialis *	0	1	1	1	1	1	1	1	1	1	1	0	1
92	V. furnissii *	1	1	1	1	1	1	1	0	1	1	0	0	1
93	V. gazogenes *	1	1	1	1	1	0	1	0	1	0	1	0	1

	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	c1	c2	c3	c4
63	0	1	0	1	0	0	0	1	1	1	0	1	1	0
64	0	1	0	1	0	0	0	1	1	1	0	1	1	0
65	0	1	0	1	1	0	1	1	1	1	0	1	1	0
66	0	0	0	1	0	0	0	1	0	0	0	0	0	0
67	0	0	0	1	0	0	0	1	0	0	0	0	0	0
68	0	0	0	1	0	0	0	1	0	0	0	0	0	0
69	0	0	0	1	0	0	0	1	1	0	0	0	0	0
70	0	0	0	1	0	0	0	1	1	0	0	0	0	0
71	0	0	0	1	0	0	0	1	1	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	1	0	0	0	1	1	0	0	0	0	0
75	0	0	0	1	0	0	0	1	1	0	0	0	0	0
76	0	0	1	1	0	0	0	1	0	0	0	0	0	0
77	0	0	0	1	0	0	0	1	1	1	0	0	0	0
78	0	1	0	1	0	0	0	1	1	1	0	1	1	0
79	0	0	0	1	0	0	0	1	1	0	0	0	0	0
80	0	0	0	1	0	0	0	1	0	0	0	0	0	0
81	0	1	1	1	0	0	0	1	1	1	0	1	1	0
82	0	0	1	1	0	0	0	1	1	0	0	0	1	0
83	0	1	1	1	0	0	0	1	1	1	0	1	1	0
84	0	0	1	1	0	0	0	1	1	1	0	1	1	0
85	0	1	1	1	0	0	0	1	1	1	0	0	1	0
86	0	1	0	1	1	0	0	1	1	0	0	1	1	0
87	0	0	0	1	0	0	0	1	0	0	0	0	1	0
88	0	1	0	1	0	0	0	1	0	1	0	0	1	0
89	0	1	1	1	0	1	0	1	1	1	0	1	1	0
90	0	0	0	1	0	0	0	0	0	0	0	0	0	0
91	0	1	1	1	1	1	1	1	1	1	0	1	1	0
92	1	1	1	1	1	1	1	1	1	1	0	1	1	0
93	0	1	1	1	0	1	0	1	1	1	0	1	1	0

	c5	c6	c7	c8	c9	c10	c11	c12	d1	d2	d3	d4	d5	d6
63	0	1	1	1	0	0	1	1	1	1	0	0	0	0
64	0	1	1	1	0	0	1	1	1	1	1	0	0	1
65	0	1	1	1	1	0	1	1	0	1	1	1	1	1
66	0	0	1	0	0	0	1	0	0	0	0	0	0	0
67	0	0	1	0	0	0	1	0	1	0	0	0	0	0
68	0	0	1	0	0	0	1	0	1	0	0	0	0	0
69	0	0	1	0	0	0	1	0	1	0	0	0	0	0
70	0	0	1	0	0	0	1	0	1	0	0	0	0	0
71	0	0	1	0	0	0	1	0	0	0	0	0	0	0
72	0	0	0	0	0	0	1	1	1	0	0	0	0	0
73	0	0	0	0	0	0	1	1	1	1	1	0	0	0
74	0	0	0	0	0	0	1	0	1	1	1	1	0	1
75	0	0	0	1	0	0	1	0	1	1	1	1	0	1
76	0	0	1	0	0	0	1	0	0	1	0	0	0	0
77	0	0	0	1	0	0	1	0	1	1	1	1	0	1
78	0	0	1	1	0	0	1	1	1	1	0	0	0	0
79	0	0	1	0	0	0	1	0	1	1	1	0	0	0
80	0	0	1	0	0	0	1	0	1	0	0	0	0	0
81	0	1	1	1	0	0	1	1	1	1	1	0	0	0
82	0	1	1	1	0	0	1	1	1	0	0	1	0	0
83	0	1	1	1	0	0	1	1	1	0	1	0	0	0
84	0	1	0	1	1	0	1	0	1	1	0	0	0	0
85	0	1	1	1	0	0	1	1	1	1	1	0	0	0
86	0	1	1	1	0	0	1	1	1	1	1	0	0	0
87	0	0	1	1	0	0	1	0	0	0	0	0	0	0
88	0	0	0	0	0	0	1	1	1	0	0	0	0	0
89	0	0	1	1	0	0	1	1	0	1	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	0	1	1	1	1	1	1	1	1	1	1	1	0	1
92	0	1	1	1	1	1	1	1	1	1	1	1	0	1
93	0	1	1	1	1	0	1	1	1	1	1	1	0	0

	d7	d8	d9	d10	d11	d12	e1	e2	e3	e4	e5	e6	e7	e8
63	1	0	0	0	1	0	0	0	0	1	0	1	1	1
64	1	0	1	0	1	0	0	0	0	1	0	1	1	1
65	1	0	1	0	1	0	0	0	0	1	0	1	1	0
66	0	0	0	0	0	0	0	1	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	1	1	0	1
68	0	0	0	0	0	0	0	0	0	0	1	1	0	1
69	0	0	0	0	0	0	0	1	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	1	0	1
71	1	0	0	0	0	0	0	0	0	0	0	1	0	0
72	0	0	0	0	1	0	0	1	0	0	1	1	0	1
73	0	0	0	0	1	0	0	0	0	1	1	1	0	1
74	1	0	1	1	1	0	0	0	1	1	0	1	1	1
75	1	0	1	0	1	0	0	0	1	1	0	1	1	1
76	1	0	0	0	0	0	0	0	0	1	0	1	0	1
77	1	0	1	1	1	0	0	0	1	1	0	1	1	1
78	1	0	0	0	1	0	0	0	0	1	0	1	0	1
79	1	0	0	0	1	0	0	1	0	1	0	1	0	1
80	1	0	0	0	0	0	0	1	0	0	1	1	0	1
81	1	0	0	1	0	0	0	0	0	0	0	1	0	1
82	1	0	0	1	0	0	0	0	1	1	0	1	0	1
83	1	0	0	0	0	0	0	0	0	1	0	1	0	0
84	0	0	0	0	0	0	0	1	0	1	0	1	0	0
85	1	0	1	1	0	0	0	0	1	1	0	1	0	1
86	1	0	0	1	0	0	0	1	0	0	0	1	0	1
87	1	0	0	0	0	0	0	0	0	0	0	1	0	0
88	0	0	0	0	0	0	0	1	0	0	0	1	0	0
89	1	0	0	0	1	0	0	0	0	0	0	1	0	1
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	1	0	1	1	1	0	0	0	1	1	0	1	0	1
92	1	1	0	1	1	0	0	1	1	1	0	1	0	1
93	1	0	1	1	0	0	0	0	1	1	0	1	1	1

	e9	e10	e11	e12	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
63	0	0	0	1	1	0	0	0	1	1	1	1	1	1
64	0	1	0	1	1	0	0	0	1	1	1	1	1	1
65	0	0	0	1	1	1	0	0	1	1	1	1	1	1
66	0	0	0	1	1	0	0	0	0	1	1	1	1	1
67	0	0	0	1	0	0	0	0	0	1	1	1	0	1
68	0	0	0	0	0	0	0	0	0	1	1	1	0	1
69	0	0	0	1	0	0	0	0	0	1	1	0	0	1
70	0	0	0	0	0	0	0	0	0	1	1	0	0	1
71	0	0	0	0	0	0	0	0	0	1	1	1	0	1
72	0	0	0	1	0	0	0	0	1	1	0	1	1	1
73	0	0	0	1	1	1	0	0	1	1	1	1	1	1
74	0	1	0	1	1	1	0	0	0	1	0	1	0	1
75	0	1	0	1	1	0	1	0	0	0	0	1	0	1
76	0	0	0	0	1	0	0	1	0	1	1	1	1	1
77	0	1	0	1	1	0	0	1	0	0	1	1	0	1
78	0	0	0	1	1	0	0	0	1	1	1	1	1	1
79	0	0	0	1	1	1	0	0	0	1	1	1	0	1
80	0	0	0	1	1	0	0	1	0	1	1	1	0	1
81	0	0	0	1	1	1	0	0	1	1	1	1	1	1
82	0	0	0	1	1	1	0	1	1	1	1	1	1	1
83	0	0	0	1	1	1	0	1	0	1	1	1	1	1
84	0	0	0	1	1	0	0	1	0	1	1	1	1	1
85	0	0	0	1	1	1	1	0	1	1	1	1	1	1
86	0	0	0	1	1	1	0	0	0	0	0	1	1	1
87	0	0	0	0	0	0	0	0	0	1	1	0	0	0
88	0	0	0	1	1	1	0	0	0	0	0	1	1	1
89	0	1	0	1	1	0	0	0	1	1	1	1	1	1
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	1	0	0	1	1	1	1	1	1	1	1	1	1	1
92	1	0	0	1	1	1	0	1	1	1	1	1	1	1
93	0	0	0	1	1	1	1	1	1	1	1	1	1	1

	f11	f12	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11	g12
63	1	1	1	0	1	0	0	1	0	0	1	1	0	1
64	1	1	1	1	1	0	1	1	0	0	1	1	0	1
65	1	1	0	0	0	0	0	1	1	0	1	1	1	1
66	0	1	0	0	1	0	0	0	0	0	1	1	0	0
67	1	1	0	0	1	0	0	0	0	0	1	1	0	0
68	1	0	0	0	1	0	0	0	0	0	1	1	0	0
69	1	1	0	0	1	0	0	0	0	0	1	1	0	1
70	1	1	0	0	1	0	0	0	0	0	1	1	0	0
71	1	1	0	0	1	0	0	0	0	0	1	1	0	0
72	0	1	0	1	1	0	0	1	0	0	1	0	0	0
73	0	1	1	1	1	0	1	1	0	0	1	0	0	1
74	0	0	0	1	1	0	0	1	0	0	0	1	0	1
75	0	0	0	0	1	0	0	1	0	0	1	0	0	0
76	1	0	0	0	1	0	0	1	0	0	1	1	0	0
77	0	1	0	0	1	0	0	1	0	0	1	1	0	1
78	1	1	0	1	1	0	0	1	0	0	1	1	0	1
79	1	1	0	0	1	0	0	1	1	0	1	1	0	0
80	1	1	0	0	1	0	0	0	0	0	1	1	0	0
81	1	1	1	0	0	1	0	1	0	0	1	1	0	0
82	1	1	1	1	1	1	1	1	0	1	1	1	0	0
83	0	1	1	0	0	0	0	1	0	0	1	1	0	0
84	1	1	0	0	1	1	1	1	0	0	1	1	0	0
85	1	1	0	1	0	0	0	1	0	1	1	1	0	0
86	0	1	0	0	0	0	0	1	0	1	1	0	0	1
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	1	1	0	0	0	0	0	1	0	0	1	1	0	0
89	1	1	0	0	1	0	1	1	0	0	1	0	0	1
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	1	1	1	0	1	1	0	1	0	1	1	1	0	1
92	1	1	1	0	1	1	0	1	0	1	1	1	0	1
93	1	1	0	0	0	0	0	1	0	0	1	1	0	1

	h1	h2	h3	h4	h5	h6	h7	h8	h9	h10	h11	h12
63	0	1	1	1	0	0	0	0	1	0	1	1
64	1	1	0	1	0	0	0	0	1	0	1	1
65	0	1	1	1	0	1	0	0	1	0	1	1
66	0	1	1	1	0	0	0	0	0	0	0	0
67	0	1	1	1	0	0	0	0	0	0	0	0
68	0	1	1	1	0	0	0	0	0	0	0	0
69	0	1	1	1	0	0	0	1	0	0	0	0
70	0	1	1	1	0	0	0	0	0	0	0	0
71	0	1	1	1	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	1	0	0	0	0	0	0	0	0	0	0	0
74	0	1	0	0	0	0	1	1	1	0	0	0
75	0	1	0	0	0	0	0	0	1	0	0	0
76	0	1	1	1	0	0	0	0	0	0	0	0
77	0	1	0	0	0	0	0	0	1	0	0	0
78	0	1	1	1	0	0	0	0	1	0	1	1
79	0	1	1	1	0	0	0	0	1	0	0	0
80	0	1	1	1	0	0	0	0	0	0	0	0
81	1	1	1	1	0	0	0	0	1	1	1	1
82	1	1	1	1	0	0	1	0	1	1	1	1
83	1	1	1	0	0	0	0	1	1	1	1	1
84	0	1	1	1	0	0	0	1	1	1	0	0
85	0	1	1	1	0	0	0	0	1	1	1	1
86	0	1	1	0	0	0	0	0	1	0	0	0
87	0	1	1	1	0	0	0	1	1	1	1	1
88	0	1	1	1	0	0	0	1	1	1	1	1
89	0	1	0	0	0	0	0	1	0	0	0	0
90	0	0	0	0	0	0	0	1	0	0	0	0
91	1	1	1	1	0	0	0	0	1	1	1	1
92	1	1	1	1	0	1	0	1	1	1	0	0
93	0	1	1	1	0	0	0	0	1	1	0	0

	strain	a2	a3	a4	a5	a6	a7	a8	a9	a1	a11	a12	b1	b2
94	V. harveyi *	1	1	1	1	1	1	1	0	0	0	1	0	1
95	V. hollisae *	0	1	1	1	1	0	1	0	1	0	0	0	1
96	V. logei *	0	1	1	1	0	0	0	0	1	0	1	0	1
97	V. marinus *	0	1	0	1	1	0	1	0	0	0	0	0	1
98	V. mediterranei *	1	1	1	1	1	1	1	0	0	0	1	0	1
99	V. metschnikovii *	0	1	1	1	1	0	1	0	0	0	0	0	0
100	V. mimicus *	0	1	1	1	1	0	1	0	0	0	0	0	1
101	V. natriegens *	0	1	1	1	1	0	1	0	1	1	1	0	1
102	V. nereis *	0	1	1	1	1	0	1	0	0	0	0	0	1
103	V. nigrapulch. *	1	1	1	1	1	1	1	0	0	0	1	0	1
104	V. ordalii *	0	1	1	0	0	0	1	0	0	0	0	0	1
105	V. orientalis *	0	1	1	1	1	0	1	0	0	0	1	0	1
106	V. parahaemo. *	1	1	1	1	1	0	1	0	1	0	0	0	1
107	L. pelagia *	1	1	1	1	1	0	1	0	0	0	1	0	1
108	V. proteolyticus *	0	1	1	1	1	0	1	0	0	0	1	0	1
109	V. salmonicida *	0	1	0	1	1	0	1	0	0	0	0	0	1
110	V. splendidus *	0	1	1	1	1	0	1	0	0	0	1	0	1
111	V. tubiashii *	0	1	1	1	1	0	1	0	0	0	1	0	1
112	V. vulnificus *	1	1	1	1	1	1	1	0	0	0	1	0	1
113	P. leiognathi *	0	1	1	1	0	1	1	0	0	0	0	0	1
114	P. angustum *	0	1	1	1	1	1	1	0	0	0	0	0	1
115	P. phosphoreum *	0	1	1	1	0	0	1	0	0	0	0	0	1
116	A. caviae *	0	1	1	1	1	0	1	0	1	0	1	0	1
117	A. hydrophilia *	0	1	1	1	1	0	1	0	1	0	0	0	1
118	A. sobria *	0	1	1	1	1	0	1	0	0	0	0	0	1
119	A. salmonicida *	0	1	1	1	1	0	0	0	0	0	0	0	1

	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	c1	c2	c3	c4
94	0	1	1	1	0	0	0	1	1	1	1	1	1	1
95	0	1	0	1	0	0	0	1	0	1	0	0	1	0
96	0	1	1	1	0	1	1	1	1	1	1	1	1	1
97	0	0	0	1	0	0	0	1	0	0	0	0	1	0
98	1	1	1	1	1	1	0	1	1	1	1	1	1	0
99	0	0	0	1	0	0	0	1	0	0	0	0	0	0
100	0	1	0	1	0	1	0	1	1	0	0	0	0	0
101	0	1	1	1	0	0	0	1	1	1	0	1	1	0
102	0	0	0	1	0	0	0	1	0	0	0	0	1	0
103	0	0	0	1	0	1	0	1	0	1	0	0	1	0
104	0	0	1	1	0	0	0	1	0	0	0	0	1	0
105	0	0	0	1	0	0	0	1	1	1	0	0	1	0
106	0	1	1	1	0	0	0	1	1	1	0	1	1	0
107	0	1	1	1	0	1	1	1	1	1	0	1	1	0
108	0	0	0	1	0	0	0	1	1	1	0	0	1	0
109	0	1	0	1	0	0	0	1	0	0	0	0	1	0
110	0	0	0	1	0	0	0	1	1	1	0	0	1	0
111	0	1	1	1	0	0	0	1	1	1	1	0	1	0
112	0	1	1	1	0	1	0	1	0	1	0	0	1	0
113	0	1	0	1	0	0	0	1	0	1	0	0	1	0
114	0	1	0	1	0	0	0	1	0	1	0	0	1	0
115	0	1	0	1	0	1	0	1	0	1	0	0	1	0
116	0	1	0	1	0	0	0	1	1	0	0	1	1	0
117	0	1	0	1	0	1	0	1	1	1	0	0	1	0
118	0	1	0	1	0	0	0	1	1	1	0	1	1	0
119	0	1	0	1	0	0	0	1	1	0	0	1	1	0

	c5	c6	c7	c8	c9	c10	c11	c12	d1	d2	d3	d4	d5	d6
94	0	1	1	1	0	0	1	1	1	1	1	1	0	0
95	0	0	1	1	1	0	1	0	1	1	0	0	0	1
96	0	1	1	0	1	0	0	1	1	0	0	0	0	1
97	0	0	0	0	0	0	1	0	1	0	0	0	0	0
98	0	1	1	1	1	0	1	1	1	1	1	1	0	0
99	0	0	1	0	0	0	0	0	0	0	0	0	0	1
100	0	1	0	1	0	0	1	0	0	0	0	0	0	0
101	1	1	1	1	0	0	1	1	1	1	1	1	0	0
102	0	0	1	1	1	0	1	0	1	0	0	0	0	0
103	0	0	1	1	1	0	1	0	0	0	0	0	0	0
104	0	0	1	0	0	0	1	0	0	0	0	0	0	0
105	0	0	1	1	0	0	1	0	0	0	0	0	0	0
106	0	1	0	1	0	0	1	1	1	0	0	0	0	0
107	0	1	1	1	0	1	1	1	1	1	0	0	0	0
108	0	1	0	1	1	1	1	1	1	1	1	1	0	0
109	0	0	0	0	0	0	1	0	0	0	0	0	0	0
110	0	1	0	1	0	0	1	1	1	0	0	0	0	0
111	0	1	1	1	0	0	1	1	1	0	0	0	0	0
112	0	0	0	1	1	0	1	1	0	0	0	0	0	0
113	0	0	0	0	0	0	1	1	1	1	0	1	0	0
114	0	0	1	0	0	0	1	1	1	0	0	0	0	0
115	0	0	0	0	0	0	1	0	0	0	0	0	0	0
116	0	0	1	1	0	0	1	1	1	1	0	0	0	0
117	0	0	1	1	0	0	1	1	1	0	0	0	0	0
118	0	0	1	1	0	0	1	1	1	1	0	0	0	0
119	0	0	1	1	0	0	1	1	0	0	0	0	0	0

	d7	d8	d9	d10	d11	d12	e1	e2	e3	e4	e5	e6	e7	e8
94	1	0	1	1	0	0	1	0	0	1	0	1	0	1
95	1	0	0	0	0	0	0	1	0	1	0	1	0	1
96	0	0	0	0	0	0	1	1	1	0	0	0	0	1
97	0	0	0	0	0	0	0	0	0	0	0	1	0	1
98	1	0	1	0	0	0	0	0	0	1	0	1	0	1
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	1	0	1	0	0	0	0	0	0	0	0	1	0	0
101	1	0	0	1	1	0	0	0	1	1	0	1	1	1
102	1	0	0	0	1	0	0	1	0	1	0	1	0	1
103	0	0	0	0	1	0	0	1	0	0	0	1	0	0
104	0	0	0	0	0	0	0	0	0	0	0	1	0	0
105	0	0	0	0	0	0	0	0	0	0	0	1	0	0
106	1	0	0	0	0	0	0	0	0	1	0	1	0	1
107	1	0	0	0	0	0	0	1	1	0	0	1	0	1
108	1	0	0	1	0	0	0	1	1	1	0	1	0	1
109	0	0	0	0	0	0	0	0	0	0	0	1	0	0
110	0	0	0	0	0	0	0	0	0	1	0	1	0	1
111	1	0	1	0	1	0	0	0	0	0	0	1	0	0
112	1	0	1	0	0	0	0	0	0	0	0	1	1	1
113	1	0	0	0	0	0	0	0	1	1	0	1	0	0
114	1	0	1	0	0	0	0	0	0	0	0	1	0	0
115	0	0	0	0	0	0	0	0	0	0	0	1	0	0
116	1	0	0	0	1	0	0	0	0	1	1	1	0	1
117	1	0	0	0	1	0	0	0	0	0	0	1	0	0
118	1	0	0	0	1	0	0	0	0	1	1	1	0	1
119	0	0	0	0	0	0	0	1	0	0	0	0	0	1

	e9	e10	e11	e12	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
94	0	0	0	1	1	1	1	1	1	1	1	1	1	1
95	0	0	0	1	1	1	0	0	0	1	1	1	1	1
96	0	0	0	0	1	0	0	0	1	0	0	0	0	0
97	0	0	0	0	0	0	0	0	1	1	1	1	0	1
98	0	0	0	1	1	1	1	1	1	1	1	1	1	1
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	1	0	1
101	1	0	0	1	1	1	0	1	1	1	1	1	1	1
102	0	0	0	1	1	0	0	1	1	1	1	1	1	1
103	0	0	0	1	0	0	0	0	0	1	1	1	0	1
104	0	0	0	0	0	0	0	1	1	0	1	1	1	1
105	0	0	0	1	1	0	0	0	1	1	1	1	1	1
106	0	0	0	1	1	1	0	0	0	0	0	1	1	1
107	0	0	0	1	1	0	0	0	1	1	1	1	1	1
108	0	0	0	1	1	1	1	1	1	1	1	1	1	1
109	0	0	0	0	0	0	0	0	1	1	1	1	0	1
110	0	0	0	1	1	0	0	1	1	1	1	1	1	1
111	0	0	0	1	1	1	1	0	1	1	1	1	1	1
112	0	0	0	1	1	1	1	1	0	1	1	1	1	1
113	0	0	0	1	1	1	0	1	0	1	1	1	1	1
114	0	0	0	1	1	1	1	0	0	1	1	1	1	1
115	0	0	0	0	0	0	0	1	0	1	1	1	1	1
116	0	0	0	1	1	1	0	0	1	1	1	1	1	1
117	0	0	0	1	1	0	0	0	0	1	1	1	1	1
118	0	0	0	1	1	1	0	0	1	1	1	1	1	1
119	0	0	0	1	1	1	0	0	0	0	1	1	1	0

	f11	f12	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11	g12
94	1	1	0	1	0	1	0	1	0	1	1	1	0	0
95	1	0	0	0	0	0	0	0	0	0	0	0	0	0
96	1	0	0	1	0	0	0	0	0	0	0	1	0	0
97	0	0	0	0	0	0	0	1	0	0	1	1	0	0
98	1	1	1	0	0	1	0	1	0	1	1	1	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	1	1	1	1	1	1	1	1	1	1	1	1	0	1
102	1	0	1	0	1	0	0	1	0	0	1	1	0	0
103	1	1	0	0	0	0	0	0	0	1	1	1	0	0
104	1	0	0	0	0	0	0	1	0	0	1	1	0	0
105	1	1	0	0	0	1	0	0	0	0	0	1	0	0
106	1	1	0	1	0	1	0	1	0	1	1	0	0	0
107	1	1	1	0	0	1	0	0	0	0	1	1	0	0
108	1	1	1	1	1	1	0	1	0	1	1	1	0	1
109	0	0	0	0	0	0	0	0	0	0	1	1	0	0
110	1	1	1	0	0	1	0	1	0	1	1	1	0	0
111	1	1	1	1	0	1	0	1	0	0	1	1	0	0
112	1	1	0	0	0	0	0	0	0	0	0	1	0	0
113	1	1	0	0	1	1	0	1	0	0	1	1	0	0
114	1	1	0	0	1	0	0	1	0	1	1	1	0	0
115	1	1	0	1	0	0	0	0	0	0	1	1	0	0
116	1	1	1	0	1	0	1	1	1	1	1	1	0	1
117	1	1	0	0	1	0	0	1	0	0	1	0	0	1
118	1	1	0	0	1	0	1	1	0	0	1	0	0	1
119	1	1	0	0	0	0	0	1	0	0	1	0	0	1

	h1	h2	h3	h4	h5	h6	h7	h8	h9	h10	h11	h12
94	1	1	1	1	0	0	0	0	0	0	1	1
95	0	1	0	1	0	0	0	0	1	0	0	0
96	0	0	0	1	0	0	0	1	1	0	0	0
97	0	1	1	0	0	0	0	0	1	1	0	0
98	1	1	1	1	0	1	0	0	1	1	1	1
99	0	1	1	0	0	0	0	0	1	0	0	0
100	0	1	1	1	0	0	0	0	1	1	0	0
101	0	1	1	1	0	1	0	0	1	1	0	0
102	1	1	1	1	0	0	0	0	1	1	1	1
103	1	1	1	0	0	0	0	0	1	0	0	0
104	0	1	1	1	0	0	0	1	0	0	0	0
105	0	1	1	1	0	0	0	0	0	0	0	0
106	0	1	1	1	0	0	0	1	1	1	1	1
107	0	1	1	1	0	1	0	0	1	1	0	0
108	1	1	1	1	0	1	0	0	1	1	1	1
109	0	1	1	0	0	0	0	0	1	1	0	0
110	0	1	1	1	0	0	0	0	1	1	0	0
111	0	1	1	1	0	0	0	0	1	1	1	1
112	0	1	1	1	0	0	0	0	1	1	1	1
113	0	1	1	1	0	0	0	0	1	1	1	1
114	0	1	1	1	0	0	0	0	1	1	1	1
115	0	1	1	1	0	0	0	0	1	1	1	1
116	1	1	1	1	0	0	0	0	0	0	0	0
117	1	1	0	0	0	0	0	0	1	1	1	1
118	1	0	0	0	0	0	0	0	1	0	1	1
119	0	1	1	1	0	0	0	0	1	1	0	0