A NUMERICAL TAXONOMIC STUDY OF VIBRIO BACTERIA FROM A SEASONALLY-COLD OCEAN

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A NUMERICAL TAXONOMIC STUDY OF *VIBRIO* BACTERIA FROM A SEASONALLY-COLD OCEAN

BY

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ABSTRACT

Numerical analysis was used to characterize 113 *Vibrio* and five *Aeromonas* strains. The *Vibrio* were comprised of 33 type and reference strains, and 80 strains isolated from the seasonally-cold coastal waters of Newfoundland. The sources of the regional cold-ocean strains were brown alga and scallops. A property shared by all of the cold-ocean strains, and only some of the type and reference strains, was an ability to grow at 4°C. Several conditions, which might affect the outcome of the study, were investigated. Based on the results obtained it was decided to use, routinely, an incubation temperature of 20°C. Tests for growth on organic compounds as sole sources of carbon and energy were incubated for three weeks rather than the more common six day period. The treatment of weak positive results as weak positive, positive, or negative was investigated also. It was decided that the general conclusions reached in the study would not be significantly altered by the interpretation of weak positive results.

Strains were divided into two categories. These were made up of strains that tested positive for the arginine dihydrolase pathway and those that tested negative. All of the arginine dihydrolase-positive regional strains were from alga and most were identified as *V. splendidus* biovar I, a species of *Vibrio* known to grow at 4°C. One arginine dihydrolase-positive strain resembled *V. diazotrophicus* or *V. aestuarianus*. Of the arginine dihydrolase-negative strains, most of the strains that

could be identified were designated *V. marinus*, although these strains also resembled the fish pathogen *V. ordalii*. The former is known to grow at 4°C and the latter is not. A small number of arginine dihydrolase-negative strains were identified as *V. cyclosites* and *V. ordalii*. Four clusters of regional strains could not be identified. These may represent new species or biovarieties. These strains, all of which grew at 4°C, clustered separately from some type strains, none of which grew at this temperature. It was concluded that some of the cold-ocean strains belonged to known species of *Vibrio*, mostly ones that can grow at cold-ocean temperatures. However, not all strains could be identified and further studies may show these to be new species or biovarieties of *Vibrio*.

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DEDICATION

I was a graduate student lucky enough to be married to a very kind computer analyst. He was patient and encouraging through the entire time of this study. He generously offered his time and expertise to handle any aspects of this work involving the computer, from numerical analysis, through the layout of the entire thesis, right down to the table of contents. Whenever help was asked for, he was always supportive and made the problems easier to solve. I couldn't have asked for better.

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INTRODUCTION

The Importance of *Vibrio* Pacini 1854, 411^{AL} Species:

Bacteria, belonging to the genus *Vibrio*, are abundant in aquatic environments. They also comprise the normal microbiota of aquatic vertebrates and invertebrates, and some cause diseases in these organisms. There has been an increased awareness of the pathogenic potential, of *Vibrio* other than *Vibrio cholerae*, to humans. Eleven species of *Vibrio* known to be potential pathogens of humans are: *V. alginolyticus*, *V. fluvialis*, *V. metschnikovii*, *V. parahaemolyticus*, *V. vulnificus* (West and Colwell 1984), *V. carchariae*, *V. damsela*, *V. furnissii*, *V. hollisae*, *V. mimicus* (Kelly *et al.* 1991), and *V. cincinnatiensis* (Brayton *et al.* 1986). Most of these *Vibrio* species are widely recognized for their roles in human intestinal infections, although some cause extraintestinal infections that range from simple wound infections to lethal septicemia (Kelly *et al.* 1991). Causative agents of diseases in marine animals include: *V. damsela* (Love *et al.* 1981), *V. ordalii* (Schiewe *et al.* 1981), *V. salmonicida* (Egidius *et al.* 1986), and *V. tubiashii* (Hada *et al.* 1984).

The abundance of pathogens in this genus underscores the importance of species identification for the genus *Vibrio*.

Taxonomy of the Genus *Vibrio*; The Early Years:

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 on cell wall curvature and negative Gram reaction. Somatic curvature, however, was not confined to the genus *Vibrio*, because it was evident in other genera such as *Pseudomonas* and *Spirillum* [cited from West and Colwell (1984)]. The term "vibrio" was, for many years, used as a vernacular name for any bacterium demonstrating a single somatic curvature.

Shewan *et al.* (1954) reported that the performance of a simple test could differentiate *Vibrio* from *Pseudomonas*. This was based on the susceptibility of *Vibrio* species to the pteridine compound 2,4-diamino-6,7-diisopropylpteridine, designated vibriostatic agent 0/129. Building on this, Shewan (1963) later provided a scheme for differentiating several genera of Gram-negative bacteria, including *Vibrio*. The tests included morphology, oxidase reaction, type of glucose utilization, and 0/129 sensitivity. Subsequently, the International Association of Microbiological Societies Subcommittee on the Taxonomy of Vibrios recommended a new description for the genus *Vibrio* (Feeley 1966). This description distinguished *Vibrio* from *Pseudomonas* and related genera based on type of glucose metabolism.

A revised scheme for identification of vibrios and related organisms was published by Bain and Shewan (1968). By this time numerical taxonomic criteria and molecular genetic studies were becoming important methodologies [cited from West

and Colwell (1984)]. These methodologies, when applied to strains of *Vibrio*, *Pseudomonas*, and *Spirillum*, indicated that there was sufficient dissimilarity among these genera to require separate family status [cited from Carney *et al.* (1975)].

The family *Vibrionaceae* was proposed by Veron (1965). The intent was to group genera which were, for the most part, then known to be oxidase-positive and motile by means of polar flagella. This differentiated them from the family *Enterobacteriaceae* which was comprised of oxidase-negative genera with peritrichous flagella (Cowan 1974a; Baumann and Schubert 1984). Members of the families *Spirillaceae* and *Pseudomonadaceae* were different from *Vibrionaceae* in that they were not facultatively fermentative when grown in glucose or other carbohydrate-containing media (Krieg and Smibert 1974). The family *Vibrionaceae*, described by Veron (1974) in the eighth edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974), included the genus *Vibrio*, with just five species, and related genera; *Aeromonas*, *Plesiomonas*, *Photobacterium*, and *Lucibacterium*. The genus *Beneckea* was included as a genus of uncertain taxonomic position (Shewan and Veron 1974).

Taxonomy of the Genus *Vibrio*; The Genera *Beneckea* and *Vibrio*:

At the time of the eighth edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974), some members of the *Vibrionaceae* were known to produce peritrichous flagella under certain conditions. These were not

included in the genus *Vibrio* but were assigned to the genus *Beneckea* (Baumann *et al.* 1971), because the definition of *Vibrio* at that time was restricted to polarly-flagellated organisms. The genus *Lucibacterium* consisted of a group of luminous marine bacteria that showed strong similarity to members of the genus *Vibrio* (Hendrie *et al.* 1970). These bacteria, however, possessed peritrichous as well as polar flagellation and were insensitive to 0/129, thus excluding them from the genus *Vibrio*. The strains described as *Lucibacterium* were then transferred to the genus *Beneckea* by Reichelt *et al.* (1976).

It was subsequently found that members of the genus *Beneckea* resembled those of the genus *Vibrio* in several major physiological and biochemical properties, as well as moles % G+C content of DNA (Baumann *et al.* 1980a). Also, it had been previously determined that, when grown on solid media, many species of *Vibrio* synthesize unsheathed peritrichous flagella (Allen and Baumann 1971). Attempts to expand the genus *Beneckea* to include strains previously assigned to *Vibrio* created confusion, rather than clarification, of the taxonomy of *Vibrionaceae* (Baumann *et al.* 1973; West and Colwell 1984). As a result, the genus *Beneckea* was abolished (Baumann *et al.* 1980a), and all strains within that genus became members of the genus *Vibrio*.

As a result of the amalgamation of the genus *Beneckea* with *Vibrio*, twenty species, including eight biovars, were assigned to the genus *Vibrio* by Baumann *et al.* (1980a). These species and biovars are listed under the genus *Vibrio* in the

determinative tables of Baumann *et al.* (1984) in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984). These *Vibrio* are assigned to the family *Vibrionaceae*, along with the genera *Aeromonas*, *Photobacterium*, and *Plesiomonas*.

Other Changes Within the Genus *Vibrio*:

During the past decade or so, with the re-evaluations of the genus *Vibrio* which have taken place, some species originally associated with this genus have been reassigned to other genera.

To illustrate this, a group of Gram-negative bacteria that demonstrated somatic curvature, but neither oxidized nor fermented sugars, were called the microaerophilic vibrios (West and Colwell 1984). These species, once designated *Vibrio fetus*, *Vibrio jejuni*, and *Vibrio sputorum*, are now placed in the genus *Campylobacter* (Smibert 1978).

An obligately anaerobic, Gram-negative rod which was unable to ferment carbohydrates was once designated *Vibrio succinogenes*. Subsequent phenotypic and DNA studies supported the reassignment of this species to the genus *Wolinella* (Tanner *et al.* 1981).

Finally, a psychrophilic, red-pigmented organism from a marine environment was designated *Vibrio psychroerythrus* by D'Aoust and Kushner (1972). This was found to be different from red-pigmented members of *Vibrio gazogenes* by Harwood (1978).

The taxonomic status of *V. psychroerythrus* was still unresolved at the time of West and Colwell (1984).

Since the abolition of the genus *Beneckea* in the early 1980's, many new *Vibrio* species have been described. This process is an ongoing one, and the taxonomy of *Vibrio* is still in a considerable state of flux (West and Colwell 1984). By 1985 there had been more than 18 major numerical taxonomic studies of the family *Vibrionaceae* performed (MacDonell and Colwell 1985).

To illustrate the rapid expansion of species to the genus *Vibrio*, the following new species, none of which were included in Baumann *et al.* (1984), have been named since 1981: *V. aestuarianus* (Tison and Seidler 1983), *V. carchariae* (Pavia *et al.* 1989), *V. cincinnatiensis* (Brayton *et al.* 1986), *V. damsela* (Love *et al.* 1981), *V. diazotrophicus* (Guerinot *et al.* 1982), *V. furnissii*, a renaming of *V. fluvialis* biovar II (Brenner *et al.* 1983a), *V. hollisae* (Hickman *et al.* 1982), *V. mediterranei* (Pujalte and Garay 1986), *V. mimicus* (Davis *et al.* 1981), *V. navarrensis* (Urdaci *et al.* 1991), *V. ordalii*, a renaming of *V. anguillarum* biovar II (Schiewe *et al.* 1981), *V. orientalis* (Yang *et al.* 1983), *V. salmonicida* (Egidius *et al.* 1986), and *V. tubiashii* (Hada *et al.* 1984).

Recent Re-evaluations of the Genus *Vibrio*:

In recent years, further changes have taken place within *Vibrio* and its family, *Vibrionaceae*. MacDonell and Colwell (1985), in their study of cluster analysis of 5S

ribosomal ribonucleic acid [rRNA] sequence data, proposed the removal of some species from the genus *Vibrio*, and reassignment to the genus *Photobacterium* and a new genus, *Listonella*. This study was based on 5S rRNA sequences because the phyiogeny of these was considered equivalent to the phylogeny of the whole bacterial cell, and one of the objectives of this study was to develop a taxonomic scheme which would reflect the phylogeny of the family *Vibrionaceae*. Among the conclusions of MacDonell and Colwell (1985) was the maintenance, within the genus *Vibrio*, of the species: *V. alginolyticus*, *V. campbellii*, *V. carchariae*, *V. cholerae*, *V. cincinnatiensis*, *V. diazotrophicus*, *V. fluvialis*, *V. gazogenes*, *V. harveyi*, *V. metschnikovii*, *V. mimicus*, *V. natriegens*, *V. nereis*, *V. parahaemolyticus*, *V. proteolyticus*, and *V. vulnificus*.

The species *V. anguillarum*, *V. pelagius*, and *V. damsela* were found to share an evolutionary history sufficiently distinct from that of the genus *Vibrio* that a separate genus designation was suggested. The new genus *Listonella* was proposed, containing the species *Listonella anguillara*, *Listonella pelagia*, and *Listonella damsela* (MacDonell and Colwell 1985).

Two other species, *Vibrio fischeri* and *Vibrio logei*, were reassigned to the genus *Photobacterium* as *Photobacterium fischeri* and *Photobacterium logei*, respectively (MacDonell and Colwell 1985).

In addition, *Vibrio marinus* was found to be significantly different from other *Vibrio* species based on 5S rRNA sequence (MacDonell and Colwell 1984), and was considered to represent a separate, new taxonomic unit. Proposal for a new taxon

was not made, however, because of the single strain status of *V. marinus* (MacDonell and Colwell 1985).

The species *V. aestuarianus*, *V. costicola*, *V. furnissii*, *V. hollisae*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. splendidus* biovars I and II, and *V. tubiashii* were not included in the study of MacDonell and Colwell (1985), so their re-evaluation according to these authors was not determined. Three other species, *V. mediterranei*, *V. navarrensis*, and *V. salmonicida* were not included in the study of MacDonell and Colwell (1985) because their descriptions were not yet published.

MacDonell and Colwell (1985) gathered data on *V. psychroerythrus*, which had never been validated as a *Vibrio* species. This organism was found, along with another, unnamed, marine strain, to share a common evolutionary history with several species of *Aeromonas*. In the same study, the *Aeromonas* species were found to be distantly related to other species in the family *Vibrionaceae*. The genus *Aeromonas* was considered to be sufficiently distantly related, to the family *Vibrionaceae*, to be excluded from this family. A new family, *Aeromonadaceae*, was proposed by Colwell *et al.* (1986).

The species *V. costicola*, *V. furnissii*, *V. nigripulchritudo*, *V. orientalis*, and *V. splendidus* biovars I and II, omitted in the study of MacDonell and Colwell (1985), were included in the genus *Vibrio* by Colwell *et al.* (1986).

Of the remaining four *Vibrio* species not included in MacDonell and Colwell (1985), *i.e. V. aestuarianus*, *V. hollisae*, *V. ordalii*, and *V. tubiashii*, three were

eventually reassigned to the newly-created genus *Listonella*. *Vibrio aestuarianus* was proposed as *Listonella aestuarianus* by Pillidge *et al.* (1987). *Vibrio ordalii* and *Vibrio tubiashii* were proposed as *Listonella ordalii* and *Listonella tubiashii*, respectively, by Pillidge and Colwell (1988). These two studies, like those of MacDonell and Colwell (1985) and Colwell *et al.* (1986), were based on 5S rRNA sequence data.

The 5S rRNA sequence method of studying relationships within the family *Vibrionaceae* was evaluated by Smith *et al.* (1991). These authors compared the relationships derived from the 5S rRNA sequence method with those determined by other methods in previous taxonomic studies. Smith *et al.* (1991) found incongruities between the results of MacDonell and Colwell (1985) and those of other taxonomic studies in which different approaches were used. These incongruities occurred at both within-genus and between-genera levels. In the opinion of Smith *et al.* (1991), these were reasons for concern about the changes in generic descriptions within *Vibrionaceae* based on 5S rRNA data. Of particular concern was the establishment of the genus *Listonella*.

In their study, Smith *et al.* (1991) considered the proposed *Listonella* species *L. anguillara*, *L. damsela*, and *L. pelagia*, and the proposed *Photobacterium* species *P. fischeri* and *P. logei*. They did not include the proposed *Listonella* species *L. aestuarianus*, *L. ordalii*, and *L. tubiashii* in their comparisons.

At the within-genus level, Smith *et al.* (1991) found several discrepancies between the results of MacDonell and Colwell (1985) and those of other workers.

For example, in contrast to the reassignment by MacDonell and Colwell (1985) of several *Vibrio* species to the genus *Listonella*, Urdaci *et al.* (1990) showed that the fatty acids profiles of three *Listonella* species, *L. anguillara*, *L. damsela*, and *L. pelagia*, were distinct and very different in a principle-components analysis. From these results they suggested that the three species represented a heterogeneous group.

The results of MacDonell and Colwell (1985) also disagreed with DNA-DNA hybridization data. *V. cholerae* and *V. mimicus*, shown to be closely related by DNA homology studies (Brenner *et al.* 1983b; Desmarchelier and Reichelt 1984), did not cluster in the 5S rRNA analyses (Smith *et al.* 1991).

There was also some question in Smith *et al.* (1991) as to the placement of *Vibrio fischeri* and *Vibrio logei* in the genus *Photobacterium* on the basis of 5S rRNA sequence. Although Baumann and Baumann (1976) originally suggested this assignment, these authors eventually placed *P. fischeri* and *P. logei* in the genus *Vibrio* (Baumann and Baumann 1981). This placement was supported by the results of enzyme studies (Baumann *et al.* 1980b).

At the between-genera level, Smith *et al.* (1991) reported that *Listonella anguillara* and *Listonella pelagia* have shown a level of DNA homology with other *Vibrio* species (Brenner *et al.* 1983b) sufficient to be considered appropriate for members of one genus (Schleifer and Stackebrandt 1983). Smith *et al.* (1991) studied the phenotypic characteristics of *L. damsela*, the other *Listonella* species they

considered. These authors concluded that, given their uncertainty about the genus *Listonella*, this species should be reassigned to the genus *Photobacterium*, and thus renamed *Photobacterium damsela*.

Rationale for the Identification Scheme Used in this Study:

Many of the *Vibrio* type species, that were included in this study, were ones that were affected by the reclassifications of MacDonell and Colwell (1985), Pillidge *et al.* (1987), and Pillidge and Colwell (1988). These proposals are duly acknowledged. However, because of the doubts about the 5S rRNA sequence data raised by Smith *et al.* (1991), the pertinent species in the present study were not named in the genera *Listonella* or *Photobacterium*. In the present study, *Vibrio* species names remain as they were at the time of Baumann *et al.* (1984), *i.e. Vibrio aestuarianus*, *V. anguillarum*, *V. damsela*, *V. fischeri*, *V. marinus*, *V. ordalii*, *V. pelagius*, and *V. tubiashii*. In support of this was a statement made in Smith *et al.* (1991):

> "Given that there is some uncertainty about the genus *Listonella* and recognizing the need for distinctive phenotypic properties at the generic level, we suggest that the characteristics of the genera *Vibrio* and *Photobacterium* as outlined in Bergey's Manual of Systematic Bacteriology (Baumann *et al.* 1984; Baumann and Baumann 1984) ... should remain as the framework for establishing generic assignments for members of the *Vibrionaceae* until enough additional substantial phylogenetic evidence and complementary phenotypic characteristics are available to warrant significant changes to the taxonomy of this family."

Identification of *Vibrio* Strains from a Cold-Ocean Environment:

Identification of *Vibrio* strains, at the species level, was an objective of the study reported in this thesis. The present study was based largely on the most recent scheme for species identification in the genus *Vibrio*, presented by Baumann *et al.* (1984), in the form of determinative tables in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984).

The bacterial strains, to be identified at the species level in this study, have been the objects of earlier studies. They were collected from two sources in the marine environment; a marine alga during several stages of decomposition, and a marine invertebrate. These strains were initially identified as members of the genus *Vibrio* by Hollohan (1980), studying the strains from alga, and by Powell (1978), studying the strains from the marine invertebrate, the scallop. Further studies of some of these strains have been reported by Hollohan (1982) and Hollohan *et al.* (1986). Hollohan *et al.* (1986), in a numerical study of the bacterial strains from alga, showed a succession of *Vibrio*.

A property shared by all of the regional strains, used in this study, was their ability to grow at 4°C. These bacteria were isolated from a region shown to support the growth of psychrophilic and psychrotrophic bacteria (Gow and Mills 1984). An objective of the study was to determine if the identification scheme of Baumann *et al.* (1984) was adequate for species identification of these cold-ocean *Vibrio* strains.

This question is relevant because the scheme of Baumann *et al.* (1984) consisted largely of mesophilic *Vibrio* species, few of which were able to grow at 4°C. In Baumann *et al.* (1984), there were only two *Vibrio* species for which all strains were able to grow at 4°C. ZoBell (1963) reported that over 90% of the marine environment [by volume] has a temperature below 5°C. Baumann and Baumann (1981) stated that it would be of considerable interest to know whether or not bacteria from a diversity of ocean habitats would differ from previously characterized mesophilic bacteria only in their relation to temperature, or whether they would constitute different species. In applying the scheme of Baumann *et al.* (1984) to cold-ocean bacteria, it was hoped that, by using known species, the question of the existence of similar cold-ocean species, or possible new species, would be addressed.

The Use of Conventional Determinative Tests:

The taxonomy of *Vibrionaceae*, as described in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984), is considered a polyphasic taxonomy (West and Colwell 1984). This is a taxonomy in which "key" characteristics are not employed *a priori*. Instead, a very wide range of characters, each equally weighted, is used to generate taxa (Colwell 1968). Commercial multitest kits were not used in this study. Such kits, using biochemical tests in microcupules, have been used extensively in clinical laboratories for rapid identification of *Enterobacteriaceae* (West and Colwell 1984). The use of these kits, with the manufacturers' recommended

preparations, to characterize *Vibrionaceae* from the marine environment has resulted in misidentifications (Davis and Sizemore 1981). West and Colwell (1984) stated that misidentifications occurred because marine isolates did not grow well in the recommended suspending fluid, which contained insufficient sodium chloride and other electrolytes. Even though the kits have improved in their reliability for identification of *Vibrio* species, with sodium chloride-supplemented solutions for organism suspensions, misidentifications are still a problem (Kelly *et al.* 1991). Following experience using several kits, M.T. Kelly stated a personal preference for conventional tube tests for *Vibrio* identification. For these reasons, the traditional tests used in the present study are justified.

Numerical Taxonomy:

Numerical taxonomic methods were first applied to bacteriology more than 25 years ago by Peter Sneath. The purpose was to allow large amounts of data to be analyzed and assessed objectively. Since then, numerical taxonomy has been applied not only to more than 100 bacterial genera, but also it continues to be applied in other biological disciplines such as botany, mycology, and zoology (Goodfellow *et al.* 1985).

Sneath and Sokal (1973) defined numerical taxonomy as "the grouping by numerical methods of taxonomic units into taxa on the basis of their character states." The principles of numerical taxonomy are derived from the writings of J.S.L.

Gilmour, and have been found to date back to the taxonomic principles of the French botanist Adanson, who worked in the eighteenth century (Sokal 1985). The Adansonian principles of numerical taxonomy, as listed in MacDonell and Colwell (1985), are:

1. Taxa should be based on many characters, *i.e.* as many test characters as possible should be observed for a large number of samples.

2. Every character is considered to be of equal importance.

3. Taxa should be defined on the basis of overall similarity of observed characters, rather than on their ancestry.

With respect to the first Adansonian principle listed here, the following criteria help define a useful numerical taxonomic study:

1. Routine characterization tests which represent a broad spectrum of the biological activities of the organisms must be selected.

2. The methods chosen must be objective and reproducible (Goodfellow *et al.* 1985).

Based on the above criteria, Colwell and Austin (1981) noted the following steps as essential to a numerical taxonomic study of bacteria:

1. Selection of strains.

2. Selection of tests.

3. Coding and arraying of test results.

4. Computer analysis of the relationships between strains and the clustering of related strains.

5. Presentation and interpretation of the results.

MacDonell and Colwell (1985) noted that internal controls, critical to all taxonomic analyses, must be chosen during the selection of strains. Colwell and Austin (1981) stated that "reference strains which have been identified and bear a scientific name should be included in a set of bacteria under study, and these known strains should, if possible, include authentic type cultures."

For numerical taxonomy, the treatment of test results must include conversion to a numerical format. The numerical format used in most instances in the present study was the binary code. Each positive result was recorded as "1", and each negative result was recorded as "0" as recommended by Colwell and Austin (1981). In the initial stages of this study, the presence of weak positive results among the original data was recognized. This "third character", so to speak, did not fit the constraints of the binary code. Until it was decided how to treat weak positive results for cluster analysis, in this study, a code of negative results as "0", weak positive results as "1", and positive results as "2" was employed. This was possible using the RECODE command option available in SPSS-X Version 4.1 Cluster package (SPSS Inc., Chicago, Illinois). An objective of this study was to determine the best way to treat weak positive results from a bacterial study for numerical analysis. This was a question not raised in previous studies.

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 high between members of the same group, and low between members of different groups. This results in a determination of the overall similarity between strains, and a hierarchical ordering of the strains according to their similarity (Hollohan 1982). The most common presentation of a numerical taxonomic study is a tree diagram, or dendrogram (Colwell and Austin 1981). Dendrograms are easily-read displays of clusters of similar strains. The clusters can then be further characterized by determining the frequency of occurrence of each character among members of the clusters. The information gathered from this facilitates the selection of discriminatory, or distinguishing, tests for each cluster of strains. MacDonell and Colwell (1985) stated that standardization of data, *i.e.* via the binary code, "requires that the investigator be able to discriminate between those data which provide significant taxonomic information and those which do not." Determination of within-cluster frequency of characters allows this.

The inclusion of type or reference strains in the study allows for the possible identification and naming of clusters. Clusters which cannot be equated with existing named taxa may represent new and not previously described taxa (Hollohan 1982).

Ward's minimum variance clustering method:

In this study, Ward's minimum variance clustering method was used. Ward's method employs a distance, or dissimilarity, coefficient which measures distances between objects, in a space defined in various ways. This measure of dissimilarity is the Euclidean distance coefficient.

Ward's method follows a series of clustering steps that begins with n clusters, each containing one object, *i.e.* strain, in this study, and it ends with one cluster containing all objects. Ward's method is an agglomerative, hierarchical technique (Romesburg 1984). At each step, it makes whichever merger of two clusters that will result in the smallest increase in the value of an index E, called the sum-of-squares index, or variance (Romesburg 1984). At each clustering step, then, all possible mergers of two clusters are tried, the value of E for each merger is computed, and the merger whose value of E is the smallest is selected. For the next clustering step, this process is repeated.

As reported in Romesburg (1984), the value of E for each tentative set of clusters is computed as follows:

1. The mean of each cluster is calculated. This is a fictitious number whose attribute value is the average of the attribute values for the objects in the cluster.

2. The differences between each object in a cluster and its cluster r. ean, *i.e.* from # 1 above, are computed.

3. Each of the differences computed in # 2 above is squared. These are added, for each cluster, giving a sum-of-squares value for each cluster.

4. The value of E is computed by adding the sum-of-squares values for all the clusters.

Romesburg (1984) stated that, using Ward's method, objects merged at previous clustering steps are never unmerged. This may lead to near-optimal rather than optimal clustering. However, because of the popularity of Ward's method for producing dendrograms with well-defined clusters, Romesburg (1984) conceded that this method is adequate for most purposes. This is discussed later in the thesis.

Objectives of This Study:

The main objectives of this study were:

- to use numerical analysis to study regional *Vibrio* strains that shared the property of growing at 4°C and to compare them with reference and type strains, many of which do not grow at 4°C.

- for type species, described since the publication of the determinative tables of Baumann *et al.* (1984), in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984), to provide information that conforms to the criteria of the tables.

- to characterize *Vibrio* that could not be described by existing classification schemes.

MATERIALS AND METHODS

Original Sources of the Strains:

There were 80 Gram-negative, fermentative bacterial strains included in this study. These had been isolated from scallops (Powell 1978), and marine brown alga (Hollohan 1980), and were part of a culture collection of marine bacteria maintained by Dr. J. Gow, Department of Biology, Memorial University of Newfoundland.

Of the 80 strains, 24 were epizoic. They were collected and isolated from the homogenized body and viscera of giant scallops *Placopecten magellanicus* [Gmelin]. The scallops were taken from Buffett Harbour, Placentia Bay, Newfoundland [Lat. 47°32'N, Long. 53°28'W] at a depth of 16 metres. The date of collection was May 25, 1977 and the water temperature was 5.8°C. The isolation and a preliminary characterization of these strains were described by Powell (1978). The remaining 56 strains were epiphytic, having been collected from fronds of the brown alga *Alaria esculenta* [Linnaeus] Greville (Hollohan 1980). These strains were from three sub-collections taken over a period during which natural decomposition was taking place. The collections were made in 1979 on June 13 [water temperature 5°C], August 15 [water temperature 6°C], and September 20 [water temperature 10°C]. The site of collection was Logy Bay, Newfoundland [Lat. 47°37'N, Long. 52°40'W] at a depth of ten metres.

In this study, the numbers for all regional strains are listed, according to their collection date and source, in Table 1. In the original studies, a higher proportion of Gram-negative, fermentative bacteria were isolated from alga than from scallops. This is the reason that there were more strains from alga than from scallops included in this study.

The 80 strains isolated from alga and scallops are called regional strains, or regional cultures, to distinguish them from type and reference cultures. The numbers assigned to the 80 strains are cross-indexed with their original strain numbers in Appendix A.

Table 1. Numbers assigned, in this study, to regional strains isolated from *A. esculenta* and *P. magellanicus*

Collection Date	Strains
from *A. esculenta*:	
June 13, 1979 [D1 sub-collection]	1-16, 47-52
August 15, 1979 [D2 sub-collection]	17-27, 53-60
September 20, 1979 [D3 sub-collection]	28-37, 61-65
from *P. magellanicus*:	
May 25, 1977	38-46, 66-80

Type and Reference Cultures:

Thirty-three type, or reference cultures belonging to the genus *Vibrio*, and four type cultures plus one reference culture, belonging to the genus *Aeromonas*, were obtained from either the American Type Culture Collection [ATCC], Rockville, Maryland, or from the Centers for Disease Control [CDC], Atlanta, Georgia. CDC cultures were kindly sent by Don Brenner of the CDC. Cultures from the CDC, such as *V. alonsis*, CDC 9067, *V. costicola*, CDC 9031, and *V. metschnikovii*, CDC 9578 were not necessarily type cultures. The CDC strain of *V. alonsis*, obtained in 1982, has not become a recognized species of *Vibrio*. It was included in this study on the assumption that it could have been validated as a species. *V. damsela*, CDC 2588 was not designated as a type culture at the time it was obtained for this study, but was subsequently named as the type strain of *V. damsela*, under the ATCC no. 33539 (ATCC Catalogue of Bacteria and Bacteriophages 1989). *A. hydrophila*, ATCC 23211, was a reference culture. Type and reference cultures are called reference cultures, strains, or species to distinguish them from the regional strains. These cultures are listed in Table 2.

When both regional strains and reference strains are discussed in this study, they are collectively called study strains, or strains.

Table 2. Reference cultures of *Vibrio* and *Aeromonas*, from the American Type Culture Collection [ATCC], and from the Centers for Disease Control [CDC]

Reference Culture	ATCC no.	CDC no.
Aeromonas caviae	15468	
Aeromonas hydrophila	14715	
Aeromonas hydrophila	23211	
Aeromonas salmonicida subsp. *masoucida*	27013	
Aeromonas sobria	43979	
Vibrio aestuarianus	35048	
Vibrio alginolyticus	17749	
Vibrio alonsis		9067
Vibrio anguillarum biovar I	19264	
Vibrio campbellii	25920	
Vibrio carchariae	35084	
Vibrio cincinnatiensis	35912	
Vibrio costicola		9031
Vibrio cyclosites	14635	
Vibrio damsela	33539*	2588
Vibrio diazotrophicus	33466	
Vibrio fischeri	7744	
Vibrio fluvialis biovar I	33809	

Table 2 continued:

Reference Culture	ATCC no.	CDC no.
Vibrio furnissii	35016	
Vibrio gazogenes	29988	
Vibrio harveyi	25919	
Vibrio hollisae	33564	
Vibrio marinus	15381	
Vibrio mediterranei	43341	
Vibrio metschnikovii		9578
Vibrio mimicus	33653	
Vibrio natriegens	14048	
Vibrio nereis	25917	
Vibrio nigripulchritudo	27043	
Vibrio ordalii	33509	
Vibrio orientalis	33934	
Vibrio parahaemolyticus	17802	
Vibrio pelagius biovar I	25916	
Vibrio proteolyticus	15338	
Vibrio splendidus biovar I	33125	
Vibrio splendidus biovar II	25914	
Vibrio tubiashii	19109	
Vibrio vulnificus	27562	

*CDC 2588 was assigned ATCC 33539.

The ATCC and CDC cultures were chosen to include most of the *Vibrio* species listed in the determinative tables of Baumann *et al.* (1984), as well as all the *Aeromonas* species (Popoff 1984) listed in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984). Of 24 *Vibrio* species and biovars listed in the determinative tables of Baumann *et al.* (1984), only four were not tested in this study. They were: *V. cholerae*, *V. pelagius* biovar II, *V. logei*, and *V. marinus*.

V. cholerae was not included as a reference culture because it is a potentially dangerous pathogen. Also, *V. cholerae* is positive for the Voges-Proskauer reaction (Baumann *et al.* 1984). During the course of the study it became apparent that the regional strains were all Voges-Proskauer negative, suggesting that none of the regional strains was *V. cholerae*. *V. pelagius* biovar II and *V. logei* were not included, because of difficulty growing them. Published results from Baumann *et al.* (1984) for these two species were not included in this study, but the results for *V. marinus* were. This was because it was evident from Baumann *et al.* (1984) that *V. pelagius* biovar II is very similar to *V. pelagius* biovar I, and *V. logei* is very similar to *V. fischeri*. The published results for the two biovars of *V. pelagius* are almost identical, as are the results for *V. logei* and *V. fischeri*. Because *V. pelagius* biovar I and *V. fischeri* were included in this study, it was decided that these were sufficiently similar to *V. pelagius* biovar II and *V. logei*, respectively, that the published results of the latter two species need not be added. The results for *V. marinus*, used in this study, were taken from Bergey's Manual (Baumann *et al.* 1984).

As many as possible of the new *Vibrio* species, described between 1984 and the present time, were also included. However, because of difficulty in cultivating it, *V. salmonicida* (Egidius *et al.* 1986) was not included. Some initial results were obtained for the ATCC strain of this species [ATCC 43839], but it could not be adequately maintained over a period of time. The description of a new species, *V. navarrensis* (Urdaci *et al.* 1991), was published in April, 1991. By this time, most of the characterization tests were completed, so it was decided not to repeat tests just to include this species.

The reference cultures in this study were used, where applicable, as positive and negative controls for the characterization tests.

In summary, 118 bacterial strains were studied. Fifty-six were regional strains from brown alga, 24 were regional strains from giant scallops, and 38 were reference cultures. Tables 1 and 2, and Appendix A, provide lists of these strains.

Media and Controls:

Complex media were prepared from dehydrated products purchased from DIFCO Laboratories [DIFCO, Detroit, Michigan], Baltimore Biological Laboratories [BBL, Baltimore, Maryland], and Oxoid [Great Britain]. To supply the proper conditions for the growth of marine bacteria, the media were rehydrated with either 75% aged natural seawater, diluted with deionized water, or Buffered Artificial Seawater [BASW]. The materials and procedures, for preparing these media, have

been described by Hollohan *et al.* (1986). British Drug Houses [Canada] Ltd. [BDH] and Fisher Scientific Company [Toronto] certified A.C.S. reagents were used to make BASW.

For routine cultivation, a yeast extract - peptone [YEP] medium was used. This medium contained 0.1% [w/v] yeast extract [DIFCO] and 0.1% [w/v] Proteose Peptone No.3 [DIFCO] prepared with either BASW, for a medium called YEPA [yeast extract - peptone - artificial], or with 75% natural seawater for YEPN [yeast extract - peptone - natural] (Hollohan *et al.* 1986). Later in the study the concentration of yeast extract and Proteose Peptone No. 3 was increased to 0.2 and 0.3%, respectively. For routine cultivation the higher substrate concentrations produced more abundant growth.

Solid media were prepared with either Oxoid Technical Agar No.3 at 1.2% [w/v] or Oxoid Purified Agar at 1.0% [w/v]. The study strains were maintained on plates and slants of YEPA or YEPN prepared with technical grade agar. Generally, technical grade agar was used in complex solid media, while purified agar was required when testing the capacity to grow on organic compounds as sole sources of carbon and energy.

Inoculation:

Bacterial suspensions used to inoculate test media were standardized, by suspending the bacteria in sterile artificial seawater [ASW] (Hollohan *et al.* 1986),

against a No.5 McFarland Nephelometer Barium Sulphate Standard (Lennette *et al.* 1980) at wavelength 600 nm using a Bausch & Lomb Spectronic 21 spectrophotometer.

Unless otherwise specified, study strains were incubated at 20°C. In all tests, results were recorded as negative [-], weak positive [w+], or positive [+].

To minimize misinterpretation of light growth, Baumann *et al.* (1984) advised including known *Vibrio* strains as positive and negative controls. In the present study, positive and negative controls were included with the tests.

All tests were carried out in duplicate.

Cluster Analysis:

For cluster analysis, Ward's method using the squared Euclidean distance measure was applied. Dendrograms were produced with the SPSS-X Version 4.1 Cluster package. This was done using a Digital VAX 8800 computer, running VMS Version 5.4.

By using cluster analysis it was determined which strains grouped into clusters and the "frequencies" within clusters were determined. "Frequencies" refers to the percentages of negative and positive results, for each test, in a cluster. For example, if there were 12 strains in a cluster, and ten strains were positive for a particular test, then the frequencies in that cluster would be 83.3% positive, and 16.6% negative, for that test.

Once all the frequencies for a cluster were determined, *i.e.* frequencies for all 91 tests in this study, then a judgement was made deciding the percentage that would indicate that all strains in a cluster would be called negative or positive, for the test. An 85% limit was chosen, whereby tests with frequency percentages equal to or greater than 85% positive were considered positive for all strains in the cluster. Likewise, tests with frequency percentages equal to or greater than 85% negative were considered negative for all strains in the cluster. Any tests for which the negative and positive percentages were less than 85%, for example, a cluster of ten strains with four strains positive and six strains negative, were shown as "d". This d designation meant that the cluster had 16-84% strains positive for the test. The adoption of such a system for the creation of determinative tables is common in bacteriology. Examples can be found in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984) and in Cowan (1974b).

Reasons for the selection of the 85% limit are given in the **Discussion** section.

Preliminary Characterization Tests:

Although many of the regional strains had been characterized by Hollohan (1982) and Hollohan *et al.* (1986), all were tested again for certain characters, especially to ensure purity of the strains, considering that they had been subcultured over several years. Although the methods for most of the preliminary characterization tests have been described either by Hollohan *et al.* (1986) or Noble *et al.* (1990), a

brief description of the tests will be given here for continuity.

Gram stain: The following modification of the Gram stain was used in this study:

Solutions required:

- Ammonium oxalate - Crystal violet [500 mL]:

Crystal violet	10 g
95% Methanol	100 mL
Ammonium oxalate, 1% [w/v] in H_2O	400 mL

- Iodine solution [500 mL]:

Iodine	5 g
Potassium Iodide	10 g
Distilled H_2O	500 mL

- *Liquor ioai fortis* [100 mL]:

Iodine	10 g
Potassium Iodide	6 g
100% Methanol	90 mL
Distilled H_2O	10 mL

- Iodine - acetone [500 mL]:

Liquor iodi fortis	17.5 mL
Acetone	482.5 mL

- Dilute Carbol Fuchsin [500 mL]:

Ziehl-Neelsen's Carbol Fuchsin	25 mL
Distilled H_2O	475 mL

Procedure for preparation of Gram stains:

1. Slide bearing inoculum covered with ammonium oxalate - crystal violet for 30 seconds.

2. Ammonium oxalate - crystal violet poured off and slide washed thoroughly with iodine solution. Slide covered with fresh iodine solution for 30 seconds.

3. Decolorization step: Iodine solution poured off and slide washed thoroughly with iodine - acetone. Slide covered with fresh iodine - acetone for 30 seconds.

4. Slide washed thoroughly with distilled H_2O.

5. Counterstain: Slide stained with dilute carbol fuchsin for 30 seconds.

6. Slide washed with distilled H_2O, blotted, and dried.

Gram reaction and morphology were noted during examination of Gram stains. The method used here is called Preston and Morrell's modification of the Gram stain. The original description was not available. The materials and methods described above were obtained from J. Gow through personal communication.

Sodium ion requirement: All regional strains and reference cultures were tested for Na^+ requirement using a medium described by Hollohan *et al.* (1986) with the following modifications: the carbon sources were 1.0% [w/v] galactose and 1.0% [w/v] potassium glutamate. Plates, solidified with purified agar, and broths of the medium were inoculated with strains suspended in sterile ASW (Hollohan *et al.* 1986) prepared without NaCl. All plates and broths were observed for growth for seven days after inoculation.

Oxidation/Fermentation: Oxidative or fermentative metabolism of glucose was tested using two methods:

1. F-1 and F-2 media, as recommended by Baumann and Baumann (1981), were used.

2. ZOF medium (Lemos *et al.* 1985) was also used. ZOF tests were observed two, three, and seven days after inoculation, according to the protocol. This medium was developed, in 1985, as a replacement for the commercially available ZoBell's MOF medium [DIFCO] that was discontinued during the early 1980's.

Initially, 95 regional strains were tested for the above characters. Strains that were not facultatively anaerobic, Gram-negative rods that required Na^+ for growth were eliminated from further study. This left a set of 80 regional strains for further study. The reference cultures in this study were also tested for these characters.

Other Characterization Tests:

The strains were further characterized on the basis of 91 tests, chosen mostly from the determinative tables of Baumann *et al.* (1984), and partially from the determinative tables of West *et al.* (1986). Of the 91 tests carried out in this study, 78 were for utilization of carbon compounds as sole sources of carbon and energy, five were growth temperature tests, one was for the presence of an exoenzyme [chitinase], two were for the presence of internal enzymes [oxidase and the enzymes

of the arginine dihydrolase system], two were simple visual tests [swarming and pigmentation], and three were for biochemical pathways [the reduction of nitrate to nitrite, the production of gas from the fermentation of D-glucose, and the Voges-Proskauer test for the presence of acetylmethylcarbinol during glucose fermentation].

Thirteen of the following characterization tests were presented as tests printed in boldface in Baumann *et al.* (1984). They comprised a proposed "core" scheme for identifying species of *Vibrio*. These tests were for: swarming, pigmentation, arginine dihydrolase, oxidase, reduction of nitrate to nitrite, production of gas from the fermentation of D-glucose, Voges-Proskauer reaction, growth at 40°C, and utilization of sucrose, cellobiose, D-gluconate, γ-*amino*butyrate, and putrescine, as sole sources of carbon and energy. These were the first tests performed on the 118 study strains, for the purpose of identification. They were done before it was decided to determine the best incubation time for carbon source utilization by the regional strains. So, for the five carbon source utilization tests among these 13 initial tests, incubation time was six days, as in Baumann *et al.* (1984). For the remaining 73 carbon source utilization tests, incubation time totalled three weeks.

Results for the 13 initial tests were compiled separately, in Appendix C, Table 1.

Generally, the methods used to characterize the strains used in this study have been described by Baumann *et al.* (1984), Hollohan *et al.* (1986), West *et al.* (1986), and Noble *et al.* (1990). For continuity, these tests are described here and include any

modifications that may have been made.

Swarming: Strains were transferred, using a sterile loop, onto plates of YEPN or YEPA (Hollohan *et al.* 1986) prepared with technical grade agar. One long streak was made with the inoculating loop. Plates were incubated, and observed at three to five days for swarming away from the original line of growth (Henrichsen 1972).

Pigmentation: Colonies were examined after growing the strains on YEPN or YEPA (Hollohan *et al.* 1986) plates prepared with purified agar. The plates had been streaked to obtain single colonies.

Arginine Dihydrolase test: In the arginine dihydrolase system, arginine is acted upon through three unique enzymes. These are: arginine deiminase, ornithine carbamoyltransferase, and carbamate kinase, and they yield ornithine and carbamoyl phosphate, the latter forming ATP via the carbamate kinase reaction (Phillips 1986). The arginine dihydrolase test was performed using the method described by Baumann and Baumann (1981). This involved a modification of the medium developed by Thornley (1960). Over an incubation period of four days, the tests were checked at two and three and four days for a difference in colour due to alkali production in the L-arginine - containing medium.

Oxidase test: The oxidase test detected the presence of cytochrome *c* as a respiratory enzyme (Blazevic and Ederer 1975). Sheets of Whatman's No.1 filter paper, treated with several drops of a 1.0% aqueous solution of tetramethyl-paraphenylene-diaminedihydrochloride in 0.2% ascorbic acid, were inoculated with growth from colonies. A platinum loop was used. Oxidase-positive strains turned the reagent purple in sixty seconds. A positive reaction generally reflected the presence of a membrane-bound, high-potential cytochrome *c* linked to an active cytochrome oxidase (Jones 1980). The result was negative if there was no reaction in sixty seconds (Skerman 1967).

Denitrification and the reduction of nitrate to nitrite: Uninoculated tubes of nitrate reduction medium were checked to make sure they had a negative reaction. This was done because contamination of glassware or reagents with nitrous oxide may give a false-positive result for reduction (Blazevic and Ederer 1975). The denitrifying abilities of all regional strains and reference cultures were tested by the methods described in Baumann and Baumann (1981). The reduction of nitrate to nitrite was tested by the starch - iodide spot test for nitrite (Skerman 1967).

Production of gas from the fermentation of D-Glucose: Gas production from the fermentation of D-glucose was observed using the F-1 medium described for the test for oxidation/fermentation and also using Phenol red glucose broth [DIFCO]

prepared with ASW (Hollohan *et al.* 1986) and containing a Durham tube. Observations for gas production were made after four days of incubation. When using F-1 medium, if gas was produced during the fermentation of D-glucose, it pushed the agar plug up the tube.

Voges-Proskauer test: The Voges-Proskauer test detected the presence of acetylmethylcarbinol, or acetoin, in the culture medium. This confirmed a butylene glycol type of glucose fermentation. The Voges-Proskauer test was performed by Barritt's method as described by Blazevic and Ederer (1975). The medium used was prepared with BASW (Hollohan *et al.* 1986) instead of distilled water.

Growth at 4°C, 20°C, 30°C, 35°C, 40°C: For these tests, the strains were grown in BASW (Hollohan *et al.* 1986) plus 0.5% [w/v] yeast extract [DIFCO]. Inoculated tubes were incubated at the different temperatures in circulating water baths, and observed at one, two, and three weeks after inoculation.

Detection of Chitinase: The procedure was described by Hollohan *et al.* (1986). For this study soluble chitin was obtained from Sigma Biochemicals Co.

Detection of Laminarinase: The procedure was described by Hollohan *et al.* (1986). The laminarin required for the medium was obtained from Calbiochem-Berhing

Corp., and was prepared from *Laminaria digitata*. This test was not included in the cluster analysis for this study, because results were obtained only for regional strains, and not for reference strains. In order to provide all the information gathered on the regional strains in this study, the data for hydrolysis of laminarin, and thus the production of laminarinase, are presented in Appendix B.

Organic compounds as sole sources of carbon and energy: All regional strains and reference cultures were tested for their capacity to utilize 78 carbon compounds as sole sources of carbon and energy. The media used to test for growth on single carbon sources were prepared by adding each compound, except the sugars, at a final concentration of 0.1% [w/v] to BASW (Hollohan *et al.* 1986) containing purified agar. Each sugar was added at a final concentration of 0.2% [w/v]. The organic compounds were either autoclaved in the BASW plus agar, or filter sterilized. For the latter, a concentrated solution of the substrate was filter sterilized [Millipore filter, 0.45 μm pore size], and then aseptically added to the BASW plus agar, which had been sterilized by autoclaving and then cooled to 45°C. The preparation and sterilization of organic compounds has been described by Palleroni and Doudoroff (1972). Table 3 provides a list of the organic compounds studied and the method by which each compound was sterilized. The warm media were poured into standard Petri dishes.

Table 3. List of organic compounds tested as sole source of carbon and energy: Method of sterilization is given in parentheses

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Carbohydrates and Sugar Derivatives [membrane filtration]:

D-Ribose, D-Melibiose, D-Galacturonate, L-Arabinose, α-Lactose, D-Gluconate, D-Xylose, D-Glucose, D-Glucuronate, Sucrose, L-Rhamnose, Salicin, D-Trehalose, D-Mannose, *n*-Acetylglucosamine, Maltose, D-Galactose, Cellobiose, D-Fructose

Aliphatic Carboxylic and hydroxyCarboxylic Acids [autoclaving]:

Acetate, *iso*Butyrate, Heptanoate, Propionate, *iso*Valerate, Valerate, DL-β-Hydroxybutyrate, Pelargonate, DL-Lactate, DL-Glycerate, Caprate, Caprylate, Caproate

Aliphatic diCarboxylic, hydroxy- and *keto*-diCarboxylic acids [autoclaving]:

Malonate, DL-Malate, Pyruvate, Succinate, Citrate, Fumarate, α-*Keto*glutarate, Glutarate, Hydroxymethylglutarate, L-Tartrate

Aliphatic triCarboxylic acids [membrane filtration]:

cis-Aconitate

Aromatic Carboxylic and substituted Carboxylic acids [membrane filtration]:

Benzoate, Phenylacetate, D-Quinate, *p*-Hydroxybenzoate

Alcohols [no sterilization required]:

Ethanol, Isopropanol

Polyalcohols and Glycols [membrane filtration]:

meso-Erythritol, D-Sorbitol, *meso*-Inositol, D-Mannitol, Glycerol*

*sterilized by autoclaving

Table 3 continued:

Aliphatic Amino acids [autoclaving]:

Glycine, L-Alanine, D-Alanine, L-Serine, L-Threonine, L-Leucine, DL-Aspartate, L-Ornithine, L-Citrulline, *γ-Amino*butyrate, *β*-Alanine, L-Valine, L-Glutamate, Asparagine

Aromatic Amino acids [membrane filtration]:

L-Histidine, L-Proline, L-Tyrosine*

*sterilized by autoclaving

N-Substituted Amino acids [membrane filtration]:

Betaine, Hippurate, Sarcosine, Adenine*

*sterilized by autoclaving

Amines [sintered glass filtration]:

Ethanolamine, Putrescine, Xanthine

The strains to be studied were grown on YEPN (Hollohan *et al.* 1986) plates until they were 24 hours old. From these cultures, standardized cell suspensions in sterile ASW (Hollohan *et al.* 1986) were made and were aseptically transferred into the wells of 24-well sterile multiwell dishes [Falcon No. 3047, from Becton Dickinson and Co., New Jersey]. Nine of the 24 available wells were used at one time. The media were then inoculated with a sterile multipoint inoculator (Lovelace and

Colwell 1968). The multipoint inoculator was sterilized between inoculation of study strains, by ethanol immersion and subsequent flaming using a Bunsen burner. Up to nine strains were used to inoculate one plate.

The maximum number of compounds tested at one time was 25. Each test series included a control medium of BASW (Hollohan *et al.* 1986) plus purified agar, which lacked a carbon and energy source. This was included to show if there was any growth because of the presence of trace nutrients. All strains were also inoculated on YEPN (Hollohan *et al.* 1986) plates in the same manner as the test media to ensure that the cultures were viable (Palleroni and Doudoroff 1972). The five carbon source tests of the initial 13 tests were examined for growth after six days incubation. The remainder of the carbon source tests and their controls were examined for growth at one, two, and three weeks after inoculation. This was done to determine the best incubation time for the study of the carbon-utilizing ability of the regional strains. All carbon source tests in this study were tests for growth on, or utilization of, the carbon compounds. These were not tests for the production of acid from the utilization of these compounds. Data to support the chosen incubation times is presented in the **Results** section. The plates were incubated in growth chambers equipped with air-circulating fans. Drying of the agar by air currents was minimized by storing the plates in racks and covering them with aluminum foil.

RESULTS

Choosing an Incubation Temperature:

The detailed results of the growth temperature studies are given in Appendix C, Table 2. However, some general comments can be made about the temperature requirements. Essentially, all regional strains were able to grow at 4 and 20°C. In the actual temperature trials strain 36 grew at 4, but not 20°C. This was not considered significant because it could be routinely cultivated at 20°C. Strain 60 was the only regional strain that did not grow at 4°C in the temperature tests. This result was also considered an anomaly because it grew at 4°C when isolated. For these reasons the regional strains were all considered to grow at 4 and 20°C. Of the reference cultures, only ten out of 38 grew at 4°C, although all grew at 20°C. Twelve of the regional strains did not grow at 30°C. Therefore 20°C was chosen as the routine incubation temperature.

Choosing an Incubation Period for Testing Growth on Carbon Compounds:

The carbon source test plates were observed for growth at one, two, and three weeks. This generated considerable data, which is recorded in Appendix D. A synopsis of some of the more significant observations, from Appendix D, is given in Table 4. After an examination of the data in this table it was decided that carbon source utilization tests should be routinely incubated for three weeks.

Table 4. A synopsis of carbon source utilization data showing that a three week incubation period was appropriate for regional and reference strains

Strain	Carbon Source	Incubation period [weeks] 1	2	3
[Regional]				
1	L-Ornithine	w+	w+	+
9	Glycine	−	w+	+
25	Caprate	−	−	+
38	L-Glutamate	−	w+	+
46	Asparagine	w+	w+	+
77	L-Serine	−	−	+
[Reference]				
14048	D-Mannose	−	w+	+
14635	D-Quinate	−	−	+
25916	Maltose	−	w+	+
27043	D-Sorbitol	−	−	+
33564	L-Tyrosine	−	w+	+
33934	DL-Malate	−	w+	+

Symbols: − means negative, w+ means weak positive, + means positive. Strains 1, 9, and 25 were strains from *Alaria esculenta*, and strains 38, 46, and 77 were strains from *P. magellanicus*. For corresponding names to ATCC numbers above, see Table 2.

For a proportion of the regional strains, results were negative, or weakly positive, at two weeks. By three weeks they were definitely positive. Of special interest was the observation that some of the reference culture results changed over the three week incubation period as well. These reference cultures did not have

readily visible positive results until the three week reading. It was not considered practical to try to observe test plates for longer than three weeks. The agar tended to dry too much when incubated for longer periods.

Some biochemical tests were incubated for the standard, or recommended, times. Examples are the Voges-Proskauer test for acetoin production and the nitrate reduction test. These tests are based on complex media in which growth is generally faster and more abundant than observed for defined media. Also, the presence or absence of growth is readily observed, and tests can be repeated if there is no growth.

Cluster Analysis:

The results of 91 tests, done in this study, were analyzed by numerical methods and dendrograms were generated. The results of 73 carbon source utilization tests were based on readings taken at three weeks incubation. These latter results were taken from Appendix D, and a new table [Appendix C, Table 4] was constructed. The results in all the tables in Appendix C were those upon which this study's dendrograms were based.

The Treatment of Weak Positive Results in the Numerical Analysis:

From the literature on cluster analysis, it is known that, if the range of values for one attribute is greater than for another, the attribute with the greater range will

carry more weight in determining similarities among objects (Romesburg 1984). In my study the objects were the 118 study strains. For tests in which weak positive results were scored, these tests would have more influence on the clustering process than others, if they remained, as such, in the data matrix. The determinative tables of Baumann *et al.* (1984) were constructed from data in which tests were not weighted. By tradition, all tests should carry the same weight and no test should be considered more important than another. This would require that weak positive results be converted to either positive or negative results. However, recent cluster analysis programmes do allow the inclusion of three states for a single test, even though this results in some tests carrying more weight than others.

To determine the effect of weak positive results on the clustering of strains in this study, three dendrograms were generated [Figs. 1, 2, and 3]. The dendrogram in Fig. 1 is the result of cluster analysis of the data matrix with weak positives scored as weak positives, *i.e.* without standardizing the data matrix. The dendrogram in Fig. 2 is the result of cluster analysis of the data matrix with weak positives scored as positives. The dendrogram in Fig. 3 is the result of cluster analysis of the data matrix with weak positives scored as negatives.

The three different treatments of weak positive results, represented in Figs. 1, 2, and 3, did not dramatically change the general clustering of reference strains and regional strains. With just a few exceptions, the clustering of reference strains remained the same in all three dendrograms, as did the clustering of regional strains.

Figure 1. Dendrogram showing numerical analysis of all study strains with weak positive data scored as weak positive

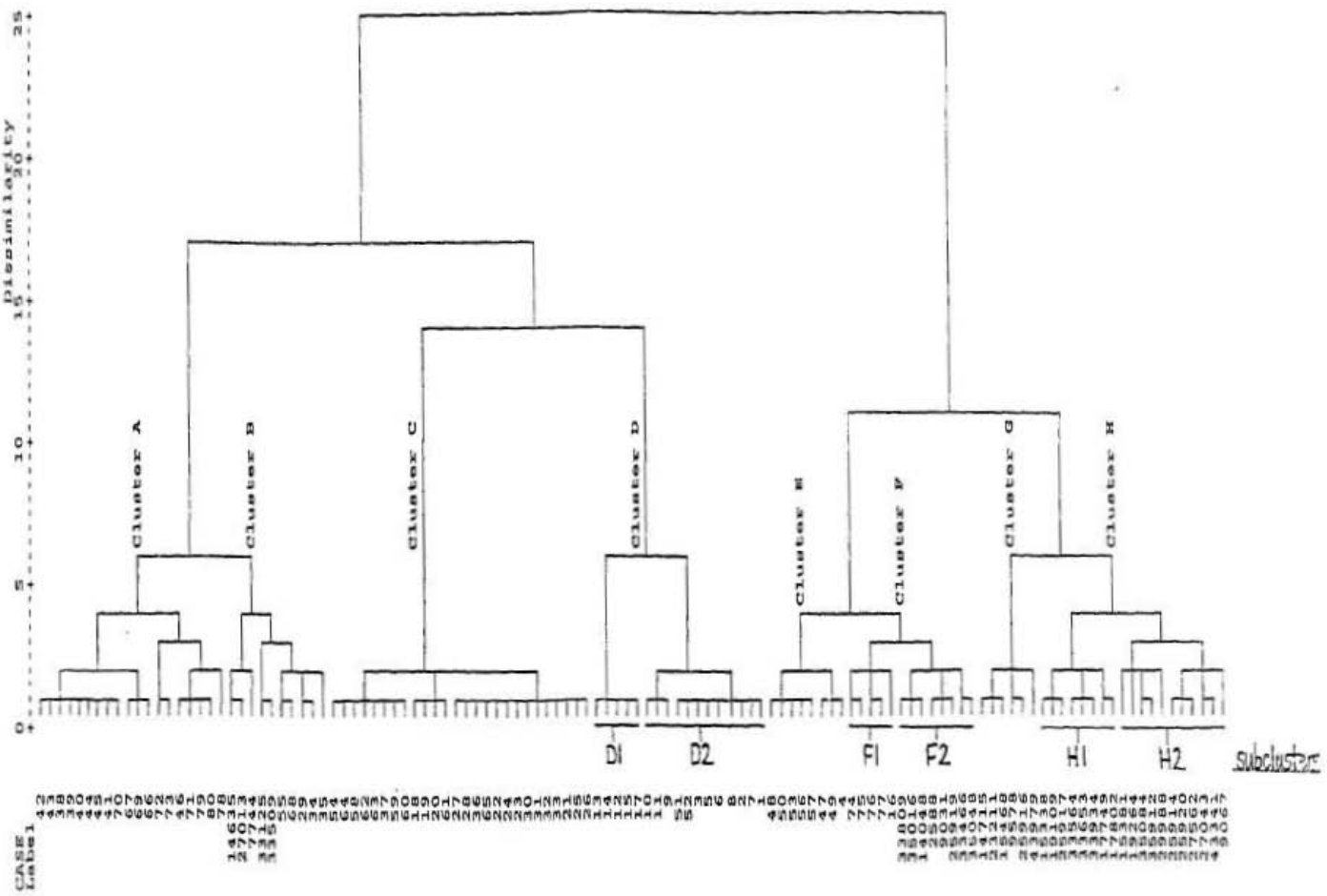
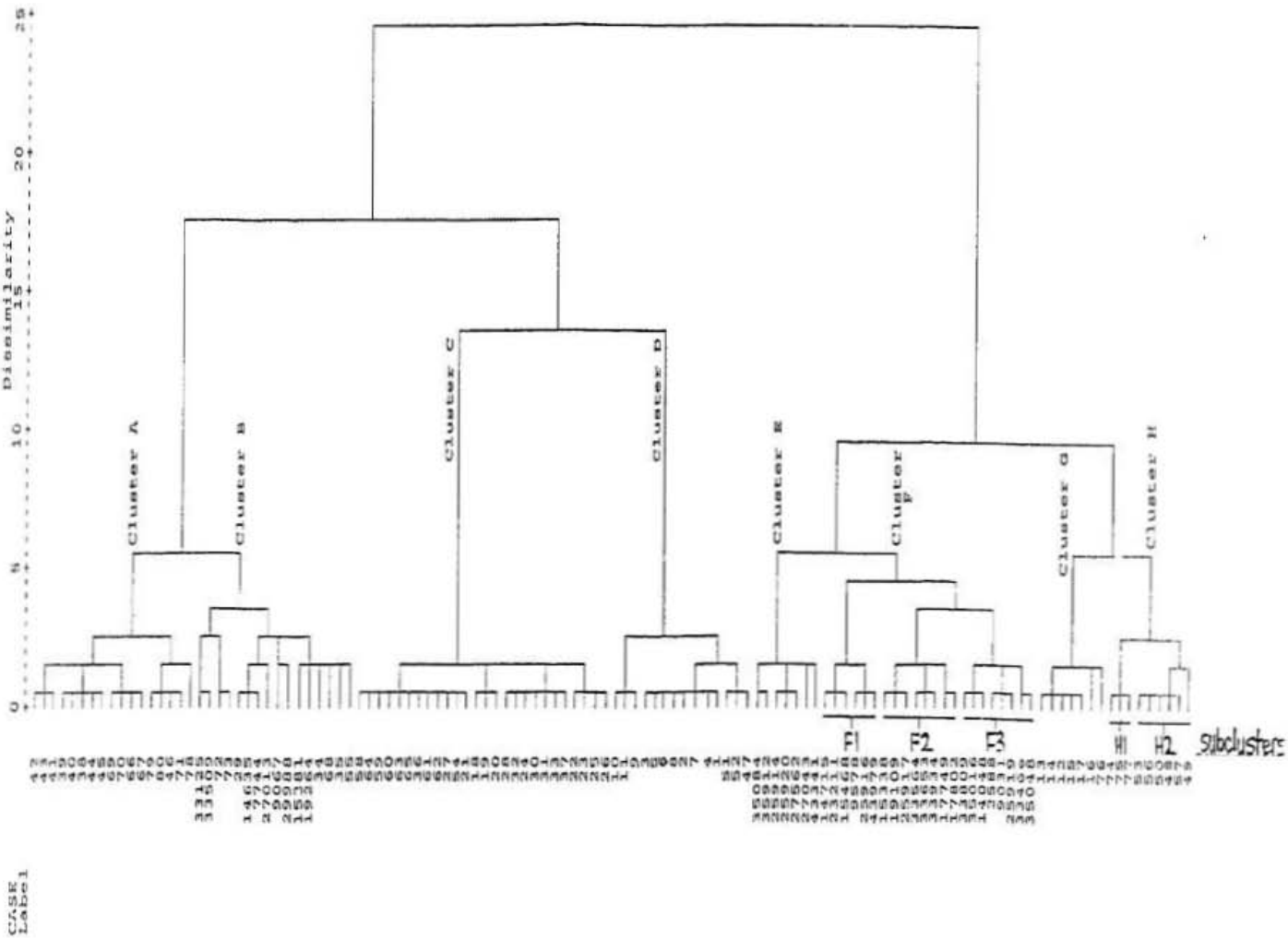

Dissimilarity
0
5
10
15
20
25
Cluster A
Cluster B
Cluster C
Cluster D
Cluster E
Cluster F
Cluster G
Cluster H
D1
D2
F1
F2
H1
H2
subclusters
CASE
Label

Figure 2. Dendrogram showing numerical analysis of all study strains with weak positive data scored as positive

Dissimilarity
0
5
10
15
20
25
Cluster A
Cluster B
Cluster C
Cluster D
Cluster E
Cluster F
Cluster G
Cluster H
H1
H2
H3
subclusters
CASE
Label

Figure 3. Dendrogram showing numerical analysis of all study strains with weak positive data scored as negative


Dissimilarity
0
5
10
15
20
25
Cluster A
Cluster B
Cluster C
Cluster D
Cluster E
Cluster F
Cluster G
Cluster H
F1
F2
F3
H1
H2
Subclusters
CASE
Label

Three differences that were evident, between clusters in Figs. 1 and 2, in which weak positives were scored as weak positives and weak positives were scored as positives, respectively, were as follows:

1. the eight reference cultures in subcluster F2, of cluster F [Fig. 1] moved, as a group, to form subcluster H2, of cluster H, in Fig. 2.

2. *V. marinus* [ATCC 15381] moved, from the cluster containing most of the reference cultures, *i.e.* the large cluster encompassing clusters G and H, in Fig. 1, to cluster B in Fig. 2.

3. Regional strain 4 moved from cluster E in Fig. 1, to cluster F in Fig. 2.

These three changes, that occurred when weak positive results were scored positive, did not appreciably affect the outcome. The main effect would be in the interpretation of the relationships between regional strains and reference strains in clusters E and F, in Fig. 1. Here, some of the regional and reference strains are more similar than they appeared to be in Fig. 2 where the reference strains mostly clustered together.

Greater changes in the structure of the dendrograms occurred when weak positives were scored negative than when they were scored positive. These differences can be seen by examining dendrograms 1 [Fig. 1] and 3 [Fig. 3]. The following changes were evident:

1. Two regional strains from scallop, 72 and 73, shifted from cluster A in Fig. 1 to cluster B in Fig. 3. This was considered a minor change.

2. *V. marinus* [ATCC 15381] shifted from the large cluster encompassing clusters G and H in Fig. 1 to cluster B in Fig. 3. This had also occurred in Fig. 2. Three other reference cultures moved, in a similar fashion, when dendrogram 3 [Fig. 3] was generated. These were: *V. alonsis* [CDC 9067], *V. gazogenes* [ATCC 29988], and *V. anguillarum* biovar I [ATCC 19264]. All four of the reference cultures that moved were from the same subcluster, H2, in dendrogram 1 [Fig. 1].

3. Two regional strains from alga, 4 and 47, moved from cluster E in Fig. 1 to cluster D in Fig. 3. This represented a major change in relationships for these two strains.

4. The remainder of cluster E in Fig. 1, *i.e.* no.'s 48, 49, 50, 53, 56, and 57, moved, as a group, to cluster H, forming subcluster H2 in Fig. 3. Three regional strains from scallop, in subcluster F1 of Fig. 1, *i.e.* no.'s 74, 75, and 77, moved to subcluster H1, thus completing cluster H in Fig. 3. It was of interest to note here that, in cluster H which consisted of strains from both alga and scallop, there was still clustering according to source of isolation. The strains from scallop were all in subcluster H1, and the strains from alga were all in subcluster H2. The general result here was to make the reference strains form discrete subclusters. This had also occurred when weak positives [Fig. 1] were changed to positive [Fig. 2].

5. Five regional strains from alga, *i.e.* no.'s 12, 13, 14, 15, and 17, in subcluster D1, and two strains, one from alga [no. 16] and one from scallop [no. 76] in subcluster F1 of Fig. 1, moved to form all of cluster G in Fig. 3. This represented

some major changes in relationships, with respect to these organisms.

6. With the exception of those four reference cultures that moved to cluster B of Fig. 3 [item no. 2 abc] the reference cultures that appeared in clusters F, G, and H of Fig. 1, formed clusters E and F in Fig. 3. It was interesting to note that: [a] the reference cultures forming subcluster F2 in Fig. 1 all moved together to form subcluster F3 in Fig. 3; [b] the reference cultures forming cluster G in Fig. 1 all moved together to form subcluster F1 in Fig. 3; [c] the reference cultures forming subcluster H1 in Fig. 1 all moved together to form subcluster F2 in Fig. 3; [d] excluding the four reference cultures that moved to cluster B in Fig. 3, the reference cultures of subcluster H2 of Fig. 1 all moved together to form cluster E of Fig. 3.

For the most part, changes that occurred because of the treatment of weak positives as negatives were between entire clusters or subclusters. There was no disintegration of clusters. Strains tended to stay within the subclusters that they occupied in either treatment. Therefore, although there was movement of subclusters when the weak positive results were scored as negative, the subclusters proved to be robust.

To summarize the comparisons among the dendrograms shown in Figs. 1, 2, and 3, it was evident that scoring weak positive results as negative, as shown in Fig. 3, altered the outcome of the cluster analysis more than when scoring the weak positive results as positive, as shown in Fig. 2. Specifically, with the weak positives called negatives, there was some increase in inter-mixing of reference strains and

regional strains. In one important aspect, the clusterings in Figs. 2 and 3 were more similar to each other than either one was to the clustering in Fig. 1. That was in the effect of producing a closer relationship among the majority of the reference cultures.

A further analysis of the treatment of weak positive results could be interesting. However, it was decided that identification could proceed if a decision was made about scoring weak positive results. The main purpose of this study was to make some tentative identifications for the regional strains, if possible. Therefore, it was decided that the dendrogram with the greatest intermixing of regional and reference strains would be studied. It was decided to use the dendrogram in Fig. 3, with weak positive results scored as negatives, as the basis for identifications made in this study. The results of this treatment were not significantly different from the results of converting weak positives to positive, but did result in a few more reference strains intermixing with regional strains outside the main clusters of reference strains. The use of three state data, as shown in Fig. 1, did give a pattern different from those of the other two treatments. This avenue would be suitable for further investigation, but was not pursued at this time.

Clustering According to Sources of the Strains:

Upon initial study, it was found that clusters formed at a dissimilarity level of about 6 in Fig. 3, were comprised of strains that could be characterized by source. Table 5 shows the sources of the strains in the clusters.

Table 5. Sources of strains comprising the clusters of dendrogram 3 [Fig. 3]

Cluster in Figure 3	Sources of strains
A	scallops
B	all sources
C	alga
D	alga
E	reference cultures
F	reference cultures
G	scallops and alga
H	scallops and alga

In more detail, the clusters can be described this way: Cluster A consisted entirely of 17 regional strains, all collected from scallop; clusters C and D were both comprised of regional strains [26 in cluster C; 14 in cluster D], collected from alga; clusters E and F were both formed by reference species [seven in cluster E; 22 in cluster F]; clusters G and H both consisted entirely of regional strains, although the strains in these clusters had been collected from both alga and scallop [in cluster G, six strains from alga and one from scallop; in cluster H, six strains from alga and three from scallop]. Cluster B represented the only instance, in Fig. 3, of regional strains and reference cultures clustering together. Here, there were nine reference cultures, four strains from alga, and three strains from scallop.

This cluster analysis showed that the regional strains, for the most part,

clustered according to their source of isolation. The majority of strains from alga [clusters C and D] clustered separately from the majority of strains from scallop [cluster A]. Only a few strains from scallop clustered with strains from alga, in clusters B, G, and H.

With respect to the clusters consisting of regional strains from *A. esculenta*, the distribution of the three sub-collections was noted. The four strains from alga in cluster B were; one from the second sub-collection [August 15, 1979, or D2], and three from the third sub-collection [September 20, 1979, or D3]. The strains in cluster C were; 14 from the D2 sub-collection and 12 from the D3 sub-collection. All the strains in cluster D were from the first sub-collection [June 13, 1979, or D1]. Cluster G strains from alga consisted of five D1 strains and one D2 strain. Strains from alga in cluster H consisted of three D1 strains and three D2 strains. This distribution showed that, for this cluster analysis, strains from the first [D1] sub-collection did not have much in common with strains from the last [D3] sub-collection. There was no clustering of D1 and D3 strains. The middle [D2] sub-collection contained strains that clustered with the D1 strains, and strains that clustered with the D3 strains. Hollohan *et al.* (1986) had already concluded that the community of vibrios on the algal fronds changed over time and so this result could be expected. Hollohan *et al.* (1986) went on to suggest that the populations were sufficiently different that they may represent three *Vibrio* species. However, these authors did not include enough reference strains to be able to attempt identification. It was an objective of the

present study to do this. With the exception of cluster B, reference strains did not group with regional strains. The regional strains in cluster B, however, had the potential of belonging to recognized species.

Reference Cultures That Were Significantly Different From Regional Cultures:

Upon analysis of the results of this study [Appendix C, Tables 1 to 4], it was found that all 80 regional strains were lacking in four out of 13 main characters highlighted by Baumann *et al.* (1984). These were swarming, colony pigment, the production of gas from the fermentation of D-glucose, and acetoin production determined by the Voges-Proskauer method. All regional strains were oxidase positive, and negative for luminescence. The test for luminescence was not done in this study. All strains were shown to be negative for luminescence by Hollohan (1982) and Hollohan *et al.* (1986) and these results were accepted as accurate for the purposes of this study. Any reference culture which is positive for one or more of the four key tests, or oxidase-negative, or luminescent, probably would not be of the same species, or biovariety, as the regional strains. This conclusion would be further strengthened if the reference culture did not fall within the same cluster as the regional strains. Based on the above criteria, 15 reference cultures could be eliminated. These, and the reasons for their elimination, are presented in Table 6. Many of the reference cultures differed by more than one character. With these reference cultures eliminated, 23 reference cultures remained.

Table 6. ATCC numbers of reference cultures eliminated as possibilities for identification, based on six traits

Traits absent in the 80 regional strains:

Swarmers	Pigmented	Luminescent	Gas Positive	V-P Positive	Oxidase Negative
				9067	
				9578	9578
			14715	14715	
15338			15338	15338	
17749				17749	
				19264	
				23211	
				27013	
	29988		29988	29988	29988
			43979	43979	
			2588		
			35016		
25917					
35084					
		7744			

For corresponding names to ATCC and CDC numbers above, see Table 2, in Materials and Methods.

Of the five reference cultures of *Aeromonas* used in this study, four were eliminated, as shown in Table 6, for their positive Voges-Proskauer reactions. Traits such as gas production, acetoin production [VP+], and the oxidase test were considered particularly significant because they represent stable, easily determined traits. The only *Aeromonas* culture remaining for possible identification with the

regional strains vs *A. caviae* [ATCC 15468].

The 80 regional strains shared some carbon source utilization properties. All utilized, as sole sources of carbon and energy, propionate, L-glutamate, succinate, fumarate, and sodium pyruvate. Four of these are aliphatic carboxylic or dicarboxylic acids. L-Glutamate is an aliphatic amino acid. None of the regional strains utilized *α-keto*glutarate or caproate, a *keto*-dicarboxylic acid and an aliphatic carboxylic acid, respectively.

Identification of Strains According to Arginine Dihydrolase Reaction:

The next step was to study, separately, all regional strains and reference cultures that were arginine dihydrolase-positive and all those that were arginine dihydrolase-negative. The rationale for this is given in the **Discussion** section. Appendix E, Tables 1 and 2 list all of the arginine dihydrolase-positive and arginine dihydrolase-negative strains, respectively, that were included in the identification process. Strains that were included in Table 6 were not included here.

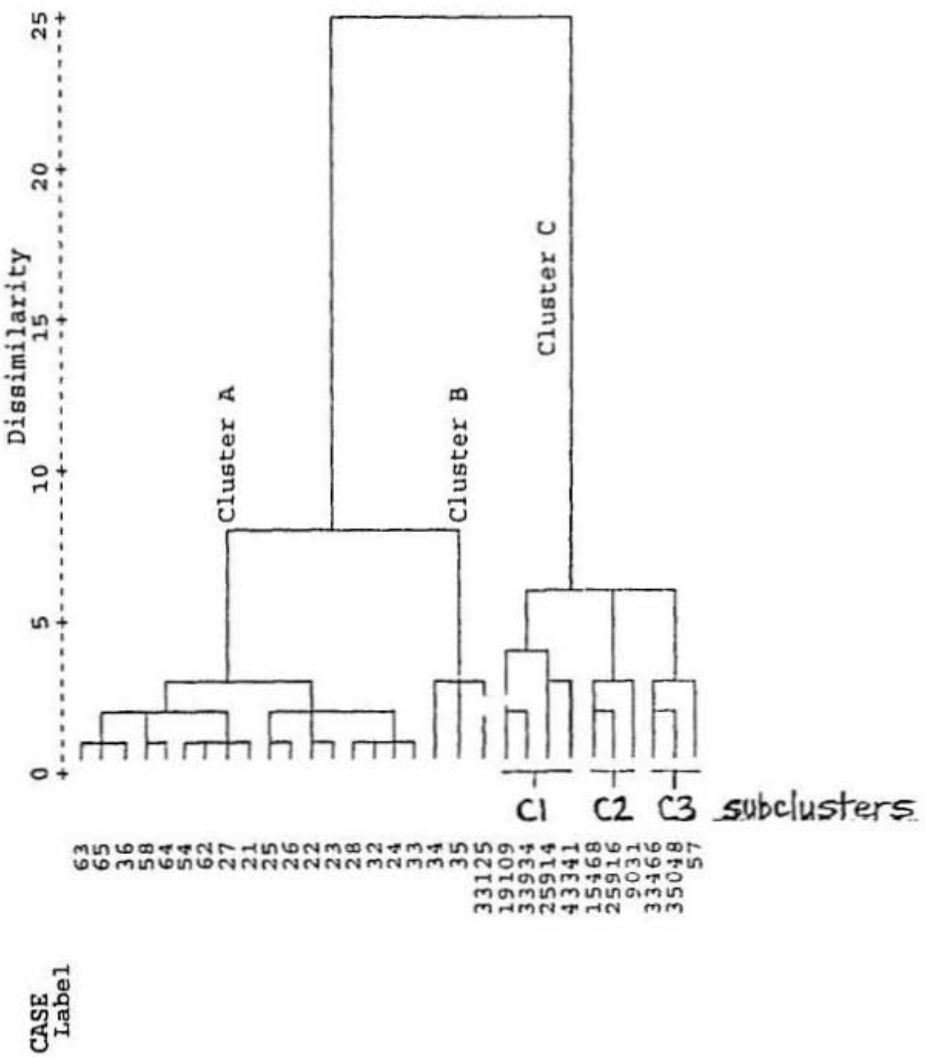
With the study strains separated according to their arginine dihydrolase reaction, each of the two groups was examined by cluster analysis, with weak positives scored as negatives. The dendrogram produced for the arginine dihydrolase-positive strains is the dendrogram shown in Fig. 4, and that for arginine dihydrolase-negative strains is shown in Fig. 5. All arginine dihydrolase-positive strains came from alga. All scallop strains and some alga strains were arginine dihydrolase-negative.

Comparison of Strains with Literature Descriptions:

In the present study, experimentally obtained data that characterized clusters was compared with published results. Generally these published descriptions were based on the properties of several strains. The data obtained was compiled and is given in Appendix F, Tables 1 to 3. It was then used to make determinative tables such as the one shown in Table 7. Here, results between the type species description and regional strains, or clusters of regional strains, were individually considered. For any test with a d result, some strains were positive while others were negative. It followed, then, that for these tests both positive and negative results among the regional strains in this study were acceptable for identification with the type species. Wherever this situation occurred, it was considered "agreement" between type species and regional strain or cluster of regional strains. A [-] symbol was assigned if less than 16% of strains in a cluster shared the character. A [d] symbol was assigned if 16-84% of strains in a cluster shared the character. A [+] symbol was assigned if greater than 84% of strains in a cluster shared the character.

It is generally acknowledged that a proportion of test results may be interpreted differently by different people, for example, those reported by people from different laboratories (Bryant *et al.* 1986). In the present study a number of reference strains were included. This served as a form of internal control because experimentally observed results should show general agreement with literature results.

Figure 4. Dendrogram showing numerical analysis of arginine dihydrolase-positive strains


Dissimilarity
0
5
10
15
20
25
Cluster A
Cluster B
Cluster C
C1
C2
C3
subclusters
CASE
Label
63
65
36
58
64
54
62
27
21
25
26
22
23
28
32
24
33
34
35
33125
19109
33934
25914
43341
15468
25916
9031
33466
35048
57

Cluster Analysis and Identification of Arginine Dihydrolase-Positive Strains:

Dendrogram 4 [Fig. 4] showed the same general separation of regional strains and reference strains as did Fig. 3. Dendrogram 4 was based on the same data matrix as dendrogram 3 [Fig. 3] except that arginine dihydrolase-negative strains, and the strains listed in Table 6, were deleted. Here again, robust clusters were maintained. All the strains forming cluster A of dendrogram 4 were the same strains as found in cluster C of dendrogram 3. As another example, all the reference strains in cluster C of dendrogram 4 were from clusters E and F of dendrogram 3.

The closest clustering of regional strains and reference species in Fig. 4 occurred in cluster B, where two regional strains clustered with one reference strain, and in subcluster C3 of cluster C, where one regional strain clustered with two reference strains.

The following summarizes each of the clusters in dendrogram 4, of Fig. 4:

Cluster A consisted entirely of 17 regional strains from alga [nine D2 strains and eight D3 strains]. The nearest reference culture to this group of regional strains was *V. splendidus* biovar I [ATCC 33125], in cluster B. A summary of the results, for strains in this cluster A, is given in Table 7. The regional strains in cluster A shared a number of characters. Sixty-five percent of the test results were 100% matches for all strains in the cluster and there were few d results. Results which were the same for all strains in the cluster, representing 100% frequency, are indicated by an asterisk in Table 7. Later, the strains in cluster A were identified as *V. splendidus*

biovar I.

Cluster B [Fig. 4] was formed by two regional strains from alga, both D3 strains, and the reference strain *V. splendidus* biovar I [ATCC 33125]. A summary of results for the strains in this cluster is also given in Table 7. For 63% of the tests there were matches. The d results were accentuated by the small number of strains in the cluster. If just one strain, of the three, had a test response different from the others, then this would elicit a d result for the cluster.

Cluster C [Fig. 4] consisted of nine reference strains and one regional strain from alga [no. 57, a D2 strain]. When the cluster was examined as subclusters, one subcluster [C3] consisted of the regional strain along with two reference strains. These were *V. diazotrophicus* [ATCC 33466] and *V. aestuarianus* [ATCC 35048]. Regional strain 57 was more similar to these two reference strains than to any other strains. A summary of results, representing all strains in cluster C, is given in Table 7. There was a high occurrence of d results for the cluster. Characters that matched made up 35% of all the tests. Cluster C of Fig. 4 was made up mostly of type species, different from each other in many respects. Important information from cluster C was that regional strain 57 appeared most similar to *V. diazotrophicus* and *V. aestuarianus*.

For the identification of the majority of arginine dihydrolase-positive regional strains in dendrogram 4 [Fig. 4], the reference strain of most interest was *V. splendidus* biovar I [ATCC 33125]. This was the reference culture most closely related

to the regional strains in clusters A and B. It appeared in the dendrogram that regional strains 34 and 35 might be more related to *V. splendidus* biovar I than the regional strains of cluster A, because these two strains were in the same cluster as the type strain. However, further analysis showed that there were few important differences between the strains in clusters A and B.

The published description of *V. splendidus* biovar I is shown for comparison with the collective results for cluster A, dendrogram 4, in Table 7. A comparison of results for the individual strains 34 and 35 of cluster B, dendrogram 4 and a description of *V. splendidus* biovar I is given in Appendix F, Table 1.

The regional strains of clusters A and B, dendrogram 4 were identified as *V. splendidus* biovar I. Table 7 gives a determinative table that shows that the majority of tests were matches for the regional strains in clusters A and B and for the type species. Of the 91 tests considered, results of cluster A and the type species differed in only 16 tests, indicating 82% similarity. The results of cluster A, dendrogram 4 which differed from the published description of *V. splendidus* biovar I are shown in boldface type in Table 7. Of the 16 cluster A results in boldface, 14 were for carbon source utilization. The exceptions were growth at 30°C and production of chitinase, for which the cluster A results were d. Also, the cluster A result was d for nine of the 14 carbon source utilization tests, indicating partial agreement.

Table 7. Determinative table of test results for clusters A, B, and C of Figure 4, based on character frequencies for each cluster. [*V. splendidus* biovar I shown for comparison with cluster A]*

Test	Cluster A	Cluster B	no. 33125 *V. splendidus* biovar I	Cluster C
Swarming	−*	−*	−	−*
Pigmentation	−*	−*	−	−*
Arginine Dihydrolase	+*	+*	+	+*
Oxidase	+*	+*	+	+*
NO_3-NO_2	+*	−*	+	+
D-Glucose gas	−*	−*	−	−*
Voges-Proskauer	−*	−*	−	−*
growth at 40°C	−*	−*	−	d
Sucrose	−	−*	d	d
Cellobiose	d	d	+	d
D-Gluconate	d	−*	d	d
γ-*Amino*butyrate	−*	−*	−	d
Putrescine	−*	−*	−	+
Chitinase	d	d	+	d
growth at 4°C	+*	d	d	d
growth at 20°C	+	+*	+	+*
growth at 30°C	d	d	+	+*
growth at 35°C	−	d	d	+
Propionate	d	+*	+	+*
DL-Malate	−*	−*	+	+
L-Serine	+*	−*	+	d
L-Alanine	+	d	+	+*
β-Alanine	−*	−*	−	d
D-Alanine	+	d	+	+*
L-Leucine	−	d	−	d
L-Tyrosine	d	d	d	+*
Malonate	−*	−*	−	d
L-Glutamate	+*	+*	+	+
L-Valine	−*	d	−	d
Succinate	+*	+*	+	+*
Fumarate	+*	+*	+	+*
L-Tartrate	−*	−*	−	−
DL-Glycerate	d	+*	d	d

Table 7 continued:

Test	Cluster A	Cluster B	no. 33125 *V. splendidus* biovar I	Cluster C
Glycine	d	−*	+	d
DL-Aspartate	+*	d	+	+
L-Ornithine	d	d	−	+
L-Citrulline	d	d	+	d
D-Xylose	−*	−*	−	d
L-Arabinose	−*	d	−	d
D-Mannose	+	d	+	d
D-Galactose	+*	d	+	d
D-Trehalose	+	d	+	+*
α-Lactose	+*	d	−	d
D-Melibiose	d	−*	−	d
D-Glucuronate	d	−*	+	d
Salicin	−*	−*	−	d
D-Galacturonate	−*	−*	−	d
Citrate	+*	d	+	+
α-*Keto*glutarate	−*	−*	+	−*
Sodium Pyruvate	+*	+*	+	+*
Ethanol	−	d	−	d
Propanol	−	d	−	d
D-Mannitol	+*	d	+	+
D-Sorbitol	−*	−*	−	d
meso-Inositol	−*	−*	−	d
p-Hydroxybenzoate	−*	−*	−	−*
L-Histidine	+	d	d	d
L-Proline	+*	d	+	+*
L-Rhamnose	−*	−*	−	−
Sarcosine	−*	−*	−	−
Betaine	−*	−*	−	d
Hippurate	−*	−*	−	d
n-Acetylglucosamine	+*	d	+	+*
D-Ribose	+	d	+	+
D-Glucose	d	d	+	d
D-Fructose	d	−*	+	+
Maltose	+	d	+	+*
Valerate	−	−*	−	d
Heptanoate	−*	−*	+	+
DL-Lactate	+*	d	+	+*

Table 7 continued:

Test	Cluster A	Cluster B	no. 33125 *V. splendidus* biovar I	Cluster C
DL-β-Hydroxybutyrate	−*	−*	−	d
Acetate	d	d	d	+
*iso*Valerate	−*	−*	−	d
*iso*Butyrate	−*	−*	−	d
Glycerol	+*	d	d	+
cis-Aconitate	+*	d	+	d
L-Threonine	+*	d	+	d
D-Quinate	−*	−*	d	−*
Benzoate	−*	−*	−	−*
Hydroxymethylglutarate	−*	−*	N/A	−*
Asparagine	+*	d	−	+*
Adenine	−	−*	−	d
Xanthine	−*	−*	−	−*
Caprate	d	−*	d	+*
Caprylate	−*	−*	−	d
Caproate	−*	−*	−	d
Ethanolamine	−*	−*	−	−*
Glutarate	−*	−*	−	d
meso-Erythritol	−*	−*	−	−*
Phenylacetate	−	−*	−	−
Pelargonate	−*	−*	−	d

*Cluster A results differing from the type species are printed in boldface.

An asterisk [*] beside a positive or negative result means that all strains in that cluster gave a positive or negative result, respectively, for the test. In other words, the character frequency in these cases was 100%.

d = 16-84% strains positive.

There were only five carbon source utilization tests that were complete mismatches with results for *V. splendidus* biovar I. It was concluded that the strains in cluster A were sufficiently similar to *V. splendidus* biovar I to be identified as this organism.

The two regional strains, 34 and 35 of cluster B, dendrogram 4 were individually compared with the published description of *V. splendidus* biovar I. The differences between strains 34 and 35, and *V. splendidus* biovar I, are summarized in Table 8. A complete comparison is given in Appendix F, Table 1. The regional strains of cluster B, dendrogram 4 were tentatively identified as *V. splendidus* biovar I for the following reasons.

As shown in Table 8, strain 34 differed from *V. splendidus* biovar I in 21 tests and was 77% similar to the type species. Strain 35 was different from *V. splendidus* biovar I in 23 tests and was 75% similar to the type species. Strains 34 and 35 were different from each other in 18 traits, as shown in Table 8, but they also had approximately the same degree of similarity to *V. splendidus* biovar I. Table 8 also shows that these two regional strains differed from the type species mostly because of different capacities to utilize certain substrates as sole source of carbon and energy. They were not as nutritionally versatile as *V. splendidus* biovar I. Regardless of these differences, strains 34 and 35 were identified as *V. splendidus* biovar I.

Table 8. Test results that differed for regional strains 34 and 35, compared with the published description of *V. splendidus* biovar I

Test	no. 33125 *V. splendidus* biovar I	no. 34	no. 35
NO_3-NO_2, Growth at 30°C, Cellobiose, DL-Malate, L-Serine, D-Alanine, Glycine, L-Citrulline, D-Glucuronate, α-*Keto*glutarate, D-Glucose, D-Fructose, Heptanoate	+	−	−
production of Chitinase, L-Alanine, DL-Aspartate, DL-Lactate, *cis*-Aconitate, L-Threonine	+	−	+
α-Lactose, Propanol	−	+	−
D-Mannose, D-Galactose, D-Trehalose, Citrate, D-Mannitol, L-Proline, *n*-Acetylglucosamine, D-Ribose, Maltose	+	+	−
Asparagine	−	−	+

The description of *V. splendidus* biovar I is that of Baumann *et al.* (1984), derived from four strains, except for the results for growth on asparagine, adenine, xanthine, and ethanolamine, which are from West *et al.* (1986), derived from eight strains.

Another comparison involving arginine dihydrolase-positive strains in this study was between regional strain 57, in subcluster C3 of dendrogram 4, and descriptions of *V. diazotrophicus* [ATCC 33466] and *V. aestuarianus* [ATCC 35048], also in subcluster C3. The conclusion in this study was that strain 57 could not yet

be identified with either of these species. In dendrogram 4 [Fig. 4] it appeared that the more similar reference culture, based on this study's results, was *V. diazotrophicus*. When compared with a literature description, compiled from results in Guerinot *et al.* (1982), West *et al.* (1986), and Bryant *et al.* (1986), strain 57 was less similar. Differences are summarized in Table 9. The lesser similarity of strain 57 was the result of minor discrepancies between this study's results and the published description of the species. Conclusions involving *V. diazotrophicus* were based on the published description. For four tests listed in Table 9, published *V. diazotrophicus* results were not available. Few published results, for the tests done in this study, could be obtained for *V. aestuarianus*, but those which were available did agree with the results from this study. This can be confirmed from results in Appendix F, Table 2. *V. aestuarianus* results obtained in this study were used in the comparison with strain 57. Overall, the results of regional strain 57 differed from the results of *V. aestuarianus* in 19 tests, indicating 79% similarity to *V. aestuarianus*, while strain 57 differed from the published description of *V. diazotrophicus* in 25 tests, indicating 73% similarity to *V. diazotrophicus*. An observation from Table 9 was that strain 57 had a slightly greater similarity to *V. aestuarianus* than to *V. diazotrophicus*, but could not be identified as either type species. The tentative conclusion was, because there were fewer differences between the regional strain and *V. aestuarianus*, that this was the species most similar to strain 57.

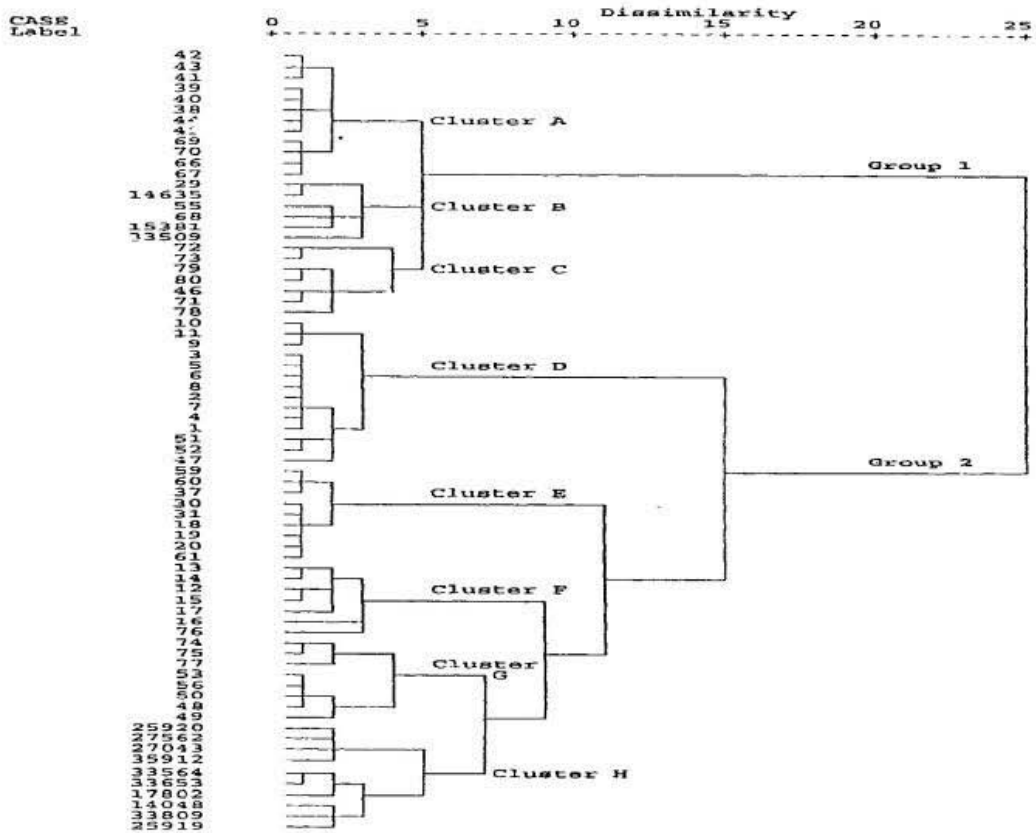
Table 9. Determinative tests for *V. aestuarianus*, the published description of *V. diazotrophicus*, and regional strain 57

Test	*V. diazotrophicus*	*V. aestuarianus*	no. 57
production of Chitinase, D-Melibiose, *meso*-Inositol, Sarcosine, Betaine, Valerate, Pelargonate	−	−	+
D-Galacturonate	+	+	−
γ-Aminobutyrate, Putrescine, Propionate, DL-Malate, L-Serine, L-Leucine, L-Tyrosine, Malonate, DL-Glycerate, Glycine, DL-Aspartate, D-Mannose, Ethanol, Propanol, DL-Lactate, L-Threonine	−	+	+
α-*Keto*glutarate	+	−	−
Growth at 40°C, D-Glucose, DL-β-Hydroxybutyrate, Acetate	+	−	+
L-Valine, D-Sorbitol, Hippurate	−	+	−
β-Alanine, *iso*Valerate, *cis*-Aconitate	N/A	−	+
L-Tartrate	N/A	+	−

N/A = not available

The published description of *V. diazotrophicus* was derived from Guerinot *et al.* (1982), West *et al.* (1986), and Bryant *et al.* (1986).

Figure 5. Dendrogram showing numerical analysis of arginine dihydrolase-negative strains


CASE
Label
Dissimilarity
0
5
10
15
20
25
Cluster A
Cluster B
Cluster C
Cluster D
Cluster E
Cluster F
Cluster G
Cluster H
Group 1
Group 2

Cluster Analysis and Identification of Arginine Dihydrolase-Negative Strains:

The dendrogram in Fig. 5 was derived from a further analysis of strains in Fig. 3 with the reference cultures, listed in Table 6, and all arginine dihydrolase-positive strains deleted. Clusters A to H of Fig. 5 still showed that regional strains and reference cultures, for the most part, clustered separately. In comparing Fig. 3 and Fig. 5, it was evident that the clusters remained robust, *i.e.* strains moved in groups, not individually, to form the new clusters in the dendrogram of Fig. 5.

In keeping with the general separation between regional strains and reference strains that has been evident in this study, all the reference cultures in Fig. 5 were found in cluster H, with the exception of three reference cultures in cluster B. In cluster B, these three clustered with three regional strains, two from alga and one from scallop.

Strains in group 1; clusters A to C of Figure 5:

The following are attributes of the clusters A, B, and C in Fig. 5. These clusters formed a group, called Group 1, that was separate from clusters D to H, found in Group 2. A determinative table showing results of tests for clusters A, B, C, and *V. marinus* is given in Table 10. The results for *V. marinus* are given because the majority of strains in Group 1 were eventually identified as *V. marinus*.

Cluster A contained 12 regional strains from scallop. The nearest reference cultures were: *V. cyclosites* [ATCC 14635], *V. marinus* [ATCC 15381], and *V. ordalii*

[ATCC 33509], in cluster B. The results in Table 10 show that 75% of the characters had 100% frequencies. These shared characters are indicated by asterisks. Also of interest was the large number of negative results for this cluster, especially in the carbon source utilization tests. This showed that these regional strains from scallop were not nutritionally versatile.

Cluster B [Fig. 5] was formed by three regional strains and three reference cultures. This was the cluster in this dendrogram where there was the closest relationship between regional strains and reference cultures. Strain 29, of the D3 sub-collection from alga, clustered most closely to *V. cyclosites* [ATCC 14635], and strains 55 [D2 sub-collection] and 68 [from scallop] clustered most closely with *V. marinus* [ATCC 15381]. Also, *V. ordalii* [ATCC 33509] was similar to these three regional strains. From Table 10, it was observed that cluster B had many d results that described characters of strains. This showed that, for many tests, the strains in the cluster did not all have the same result. Although these regional strains and reference species were placed in the one cluster, the grouping was not based on a large occurrence of matched results. Asterisks, representing shared characters for all strains in the cluster, were present for only 42% of the tests.

Cluster C [Fig. 5] consisted entirely of seven regional strains from scallop. The nearest reference cultures to this group of strains were the three in cluster B.

Table 10. Determinative table of test results for clusters A, B, and C of Figure 5, based on character frequencies for each cluster. [*V. marinus* is shown for comparisons]

Test	Cluster A	Cluster B	Cluster C	no. 15381 *V. marinus*
Swarming	−*	−*	−*	−
Pigmentation	−*	−*	−*	−
Arginine Dihydrolase	−*	−*	−*	−
Oxidase	+*	+*	+*	+
NO_3-NO_2	+*	d	d	+
D-Glucose gas	−*	−*	−*	−
Voges-Proskauer	−*	−*	−*	−
growth at 40°C	d	d	d	−
Sucrose	d	d	d	−
Cellobiose	d	d	−	−
D-Gluconate	−	d	−	+
γ-*Amino*butyrate	−*	d	−	−
Putrescine	d	−*	−*	−
Chitinase	d	d	−*	+
growth at 4°C	+*	d	+*	+
growth at 20°C	+*	+*	+*	+
growth at 30°C	+*	d	+*	−
growth at 35°C	d	d	d	−
Propionate	+*	d	+*	−
DL-Malate	−*	d	−*	+
L-Serine	+	d	d	+
L-Alanine	d	d	d	−
β-Alanine	−*	−*	−	−
D-Alanine	−*	d	d	+
L-Leucine	d	d	d	−
L-Tyrosine	d	d	d	−
Malonate	−*	−*	−*	−
L-Glutamate	+*	d	+*	+
L-Valine	−*	d	−	−
Succinate	+*	d	+*	+
Fumarate	+*	d	+*	+
L-Tartrate	−*	−*	−	−
DL-Glycerate	d	d	+	−
Glycine	−*	d	−	−

Table 10 continued:

Test	Cluster A	Cluster B	Cluster C	no. 15381 *V. marinus*
DL-Aspartate	d	d	d	+
L-Ornithine	−*	d	−	−
L-Citrulline	−*	d	−*	−
D-Xylose	−	−*	d	−
L-Arabinose	−*	−*	d	−
D-Mannose	−*	d	−*	−
D-Galactose	−	d	d	+
D-Trehalose	−*	d	−*	−
α-Lactose	−*	−*	−*	−
D-Melibiose	−*	−*	−*	−
D-Glucuronate	−*	−*	−*	−
Salicin	−*	−*	−*	−
D-Galacturonate	−*	−*	−*	−
Citrate	−*	d	d	−
α-*Keto*glutarate	−*	d	−*	+
Sodium Pyruvate	+*	d	+	−
Ethanol	−*	d	−	−
Propanol	−*	d	−*	−
D-Mannitol	d	d	d	−
D-Sorbitol	−*	−*	−*	−
meso-Inositol	−*	−*	−	−
p-Hydroxybenzoate	−*	−*	−*	−
L-Histidine	−*	d	d	−
L-Proline	−*	d	d	+
L-Rhamnose	−*	−*	−*	−
Sarcosine	−*	−*	−*	−
Betaine	−*	−*	−*	−
Hippurate	−*	−*	−	−
n-Acetylglucosamine	+*	d	d	+
D-Ribose	d	d	−*	+
D-Glucose	+*	d	+*	+
D-Fructose	+*	d	−	+
Maltose	+*	d	+*	+
Valerate	d	d	+*	−
Heptanoate	d	−*	d	−
DL-Lactate	+*	d	+*	+
DL-β-Hydroxybutyrate	−*	−*	d	−
Acetate	d	d	+	+

Table 10 continued:

Test	Cluster A	Cluster B	Cluster C	no. 15381 *V. marinus*
*iso*Valerate	−*	d	+*	−
*iso*Butyrate	−*	−*	−*	−
Glycerol	−*	d	d	+
cis-Aconitate	−*	d	d	−
L-Threonine	d	d	d	+
D-Quinate	−*	d	d	−
Benzoate	−*	−*	d	
Hydroxymethylglutarate	−*	−*	d	−
Asparagine	d	d	d	+
Adenine	−*	−*	−*	−
Xanthine	−*	−*	−*	−
Caprate	+	d	+	+
Caprylate	−*	−*	−	−
Caproate	−*	−*	−*	−
Ethanolamine	−*	−*	−*	−
Glutarate	−*	d	d	−
meso-Erythritol	−*	−*	−*	−
Phenylacetate	−*	−*	−*	−
Pelargonate	−*	−*	−	−

V. marinus results were from Baumann *et al.* (1984).

An asterisk [*] beside a positive or negative result means that all strains in that cluster gave a positive or negative result, respectively, for the test. In other words, the character frequency in these cases was 100%.

d = 16-84% strains positive.

From Table 10 it was observed that strains in cluster C did not share as many matches as did strains in cluster A. The strains from scallop making up this cluster had shared results for approximately half of the tests.

The published descriptions of *V. cyclosites*, *V. marinus*, and *V. ordalii* were obtained and compared with the determinative results for clusters A and C [Fig. 5], from Table 10. The comparisons are shown in Appendix F, Table 3, and summarized

in Tables 11 and 12. The descriptions of *V. cyclosites*, *V. marinus*, and *V. ordalii* were compared also to individual results of the three regional strains in cluster B, dendrogram 5. These comparisons are shown in Table 13. The regional strains comprising clusters A and C of dendrogram 5 were identified as *V. marinus*. For the three regional strains in cluster B of dendrogram 5, *i.e.* no.'s 29, 55, and 68; strain 29 was identified as *V. cyclosites*, and strains 55 and 68 were identified as *V. ordalii*. The analyses which led to these tentative identifications are described here.

Table 11 shows the differences between the regional strains in cluster A of dendrogram 5 and the descriptions of the three species in cluster B, dendrogram 5. Where no result is shown for a type species, then it was the same as that of the cluster of regional strains. Table 11 showed that the regional strains of cluster A differed completely from *V. cyclosites* in 15 tests, indicating 84% similarity. Of these tests, one was for growth at 4°C, and the rest were carbon source utilization tests. Complete differences were observed between cluster A and *V. marinus* in ten tests. These were nine carbon source utilization tests and one growth temperature test, for 30°C. The similarity between cluster A and *V. marinus* was 89%. Between cluster A and *V. ordalii*, complete differences occurred in 12 tests. Of these 12 tests, two were for growth at 4 and 30°C, and one was the test for nitrate reduction. The rest were carbon source utilization tests. Cluster A was 87% similar to *V. ordalii*.

Table 11. Test results of cluster A, dendrogram 5 that differed from those for *V. cyclosites*, and those in published descriptions of *V. marinus* and *V. ordalii*. [For category definitions, see footnote at end of table]

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	Cluster A
Category 1 tests:				
D-Quinate	+			−*
Betaine	w+			−*
L-Glutamate, D-Glucose, Maltose, *n*-Acetylglucosamine, D-Fructose	−			+*
Category 2 tests:				
D-Gluconate, D-Galactose		+		−
DL-Malate, D-Alanine, *α-Keto*glutarate		+		−*
Category 3 tests:				
Citrate			+	−*
NO_3-NO_2			−	+*
Category 4 tests:				
Sodium pyruvate	−	−		+*
Category 5 tests:				
L-Proline, Glycerol		+	+	−*
Growth at 30°C		−	−	+*
Category 6 tests:				
Growth at 4°C, Succinate, Fumarate, DL-Lactate	−		−	+*
L-Serine, Caprate	−		−	+

Table 11 continued:

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	Cluster A
Category 7 tests: Propionate	–	–	–	+*
Category 8 tests: Growth at 40°C, Cellobiose, Putrescine, L-Alanine, L-Leucine, L-Tyrosine, D-Mannitol, Valerate, Heptanoate	–	–	–	d
Sucrose	–	–	+	d
Growth at 35°C	+	–	–	d
production of Chitinase	–	+	d	d
DL-Glycerate	–	–	d	d
DL-Aspartate, D-Ribose, Acetate, L-Threonine	–	+	+	d
Asparagine	–	+	N/A	d

N/A = not available. d = 16-84% strains positive.

Category 1: cluster differed from *V. cyclosites* only. Category 2: cluster differed from *V. marinus* only. Category 3: cluster differed from *V. ordalii* only. Category 4: cluster differed from *V. cyclosites* and *V. marinus*. Category 5: cluster differed from *V. marinus* and *V. ordalii*. Category 6: cluster differed from *V. cyclosites* and *V. ordalii*. Category 7: cluster differed from all three species. Category 8: cluster differed partially from all three species.

An asterisk [*] beside a positive or negative result means that all strains in that cluster gave a positive or negative result, respectively, for the test. In other words, the character frequency in these cases was 100%.

Cluster A was considered most similar to *V. marinus* because of its 89% similarity to this species, and because *V. marinus* and all the regional strains in cluster A grew at 4°C [Table 11, Category 6]. Cluster A was a little less similar, *i.e.* 84%, to *V. cyclosites*, and besides the carbon source utilization tests it differed in only one growth temperature. This growth temperature difference, however, was at 4°C [Table 11, Category 6]. In dealing with the cold-ocean regional strains here, it was considered important that a similar type species grew at 4°C. Although *V. ordalii* had an 87% similarity to cluster A, it differed from cluster A in growth at 30 and 4°C [Table 11, Categories 5 and 6], and in the nitrate reduction test [Table 11, Category 3]. Based on these observations, strains in cluster A were identified as *V. marinus*.

Table 12 shows the differences between the regional strains in cluster C of dendrogram 5 and the descriptions of the three species in cluster B, dendrogram 5. Where no result is shown for a type species, then it was the same as that of the cluster of regional strains. Table 12 showed that the regional strains of cluster C differed completely from *V. cyclosites* in 15 tests, one of which was for growth at 4°C. The rest were carbon source utilization tests. Cluster C was 84% similar to *V. cyclosites*. Cluster C differed completely from *V. marinus* in 12 tests. One of these was for growth at 30°C, another for the production of chitinase, and the rest were carbon source utilization tests. Cluster C and *V. marinus* were 87% similar. Cluster C and *V. ordalii* had completely different results in 11 tests, two of which were for growth at 4 and 30°C. The other nine were carbon source utilization tests.

Table 12. Test results of cluster C, dendrogram 5 that differed from those for *V. cyclosites*, and those in published descriptions of *V. marinus* and *V. ordalii*. [For category definitions, see footnote at end of table]

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	Cluster C
Category 1 tests:				
Betaine	w+			−*
L-Glutamate, D-Glucose, Maltose	−			+*
Acetate	−			+
Category 2 tests:				
D-Gluconate		+		−
production of Chitinase, DL-Malate, *α-Keto*glutarate		+		−*
Category 3 tests‡:				

Category 4 tests:				
DL-Glycerate, Sodium pyruvate	−	−		+
Category 5 tests:				
D-Fructose		+	+	−
D-Ribose		+	+	−*
Growth at 30°C		−	−	+*
Category 6 tests:				
Growth at 4°C, Succinate, Fumarate, DL-Lactate	−		−	+*
Caprate	−		−	+

Table 12 continued:

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	Cluster C
Category 7 tests:				
Propionate, Valerate, *iso*Valerate	–	–		+ *
Category 8 tests:				
Growth at 40°C, L-Alanine, L-Leucine, L-Tyrosine, D-Xylose, L-Arabinose, D-Mannitol, Heptanoate, Glutarate, DL-β-Hydroxybutyrate, Benzoate	–	–	–	d
NO_3-NO_2	+	+	–	d
Sucrose, Citrate	–	–	+	d
Growth at 35°C, D-Quinate	+	–		d
L-Serine, D-Alanine, D-Galactose	–	+	–	d
DL-Aspartate, L-Proline, Glycerol, *n*-Acetylglucosamine, L-Threonine	–	+	+	d
L-Histidine, *cis*-Aconitate	–	–	d	d
Hydroxymethylglutarate	–	–	N/A	d
Asparagine	–	+	N/A	d

N/A = not available. d = 16-84% strains positive.

Category 1: cluster differed from *V. cyclosites* only. Category 2: cluster differed from *V. marinus* only. Category 3: cluster differed from *V. ordalii* only. In this comparison, no results fit Category 3. Category 4: cluster differed from *V. cyclosites* and *V. marinus*. Category 5: cluster differed from *V. marinus* and *V. ordalii*. Category 6: cluster differed from *V. cyclosites* and *V. ordalii*. Category 7: cluster differed from all three species. Category 8: cluster differed partially from all three species.

An asterisk [*] beside a positive or negative result means that all strains in that cluster gave a positive or negative result, respectively, for the test.

Cluster C was 88% similar to *V. ordalii*.

Because of the nearly identical similarity percentages. for cluster C. to *V. marinus* and *V. ordalii*, it was difficult to determine the species to which the cluster of regional strains was more similar. It was concluded that cluster C would be considered more similar to *V. marinus*, because *V. marinus* grows at 4°C and *V. ordalii* does not [Table 12, Category 6]. Based on these observations, strains in cluster C, like cluster A, were identified as *V. marinus*.

The number of regional strains in cluster B [Fig. 5] was small enough that it was decided to compare the test results of these strains individually with the descriptions of the reference cultures. Therefore, the actual test results of strains 29, 55, and 68, from Appendix C, Tables 1 to 4, were used in Table 13, the table showing all the comparisons with *V. cyclosites*, *V. marinus*, and *V. ordalii*.

The tentative identification for strain 29 was made using the following observations of Table 13. Strain 29 differed from *V. cyclosites* in just 11 of the 91 tests performed. Thus, there was 88% similarity between these two strains. Three of the 11 tests in which differences were observed were growth temperature tests, for 4°C, 30°C, and 35°C. The rest were carbon source utilization tests. Strain 29 differed from *V. marinus* in 23 tests. All of these were carbon source utilization tests except one, the test for production of chitinase. There was 75% similarity between strain 29 and *V. marinus*. Strain 29 differed from *V. ordalii* in 16 tests, indicating 82% similarity.

Table 13. Comparisons of regional strains in cluster B of dendrogram 5 [Fig. 5] with descriptions of *V. cyclosites*, *V. marinus*, and *V. ordalii*

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	no.29	no.55	no.68
Swarming	−	−	−	−		
Pigmentation	−	−	−	−		
Arginine Dihydrolase	−	−	−	−		
Oxidase	+	+	+	+	+	+
NO_3-NO_2	+	+	−	+		
D-Glucose gas	−	−	−			
Voges-Proskauer	−	−	−			
growth at 40°C	−	−	−		−	−
Sucrose	−	−	+	−		
Cellobiose	−	−	−	−	+	
D-Gluconate	−	+	−		+	
γ-*Amino*butyrate	−	−	−	−	+	
Putrescine	−	−	−			
growth at 4°C	−	+	−	+	+	+
growth at 20°C	+	+	+	+	+	+
growth at 30°C	+	−	−	−	+	+
growth at 35°C	+	−	−	−		
Chitinase	−	+	d	−		
Propionate	−	−	−	+	−	+
DL-Malate	−	+	−	−		
L-Serine	−	+	−	−	−	
L-Alanine	−	−	−	−		+
β-Alanine	−	−	−	−		
D-Alanine	−	+	−	−	+	+
L-Leucine	−	−	−	−	−	+
L-Tyrosine	−	−	−		+	+
Malonate	−	−	−			
L-Glutamate	−	+	+	+	+	+
L-Valine	−	−	−	−	−	−
Succinate	−	+	−	+	+	+
Fumarate	−	+	−	−	+	+
L-Tartrate	−	−	−	−		−

Table 13 continued:

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	no. 29	no. 55	no. 68
DL-Glycerate	–	–	d	–	–	–
Glycine	–	–	–	–	–	–
DL-Aspartate	–	+	+	–	+	+
L-Ornithine	–	–	–	–	–	–
L-Citrulline	–	–	–	–	–	–
D-Xylose	–	–	–	–	–	–
L-Arabinose	–	–	–	–	–	–
D-Mannose	–	–	–	+	–	+
D-Galactose	–	+	–	–	+	+
D-Trehalose	–	–	–	–	+	–
α-Lactose	–	–	–	–	–	–
D-Melibiose	–	–	–	–	–	–
D-Glucuronate	–	–	–	–	–	–
Salicin	–	–	–	–	–	–
D-Galacturonate	–	–	–	–	–	–
Citrate	–	–	+	–	+	–
α-*Keto*glutarate	–	+	–	–	–	–
Sodium Pyruvate	–	–	+	+	+	+
Ethanol	–	–	–	–	–	–
Propanol	–	–	–	–	–	–
D-Mannitol	–	–	–	–	+	+
D-Sorbitol	–	–	–	–	–	–
meso-Inositol	–	–	–	–	–	–
p-Hydroxybenzoate	–	–	–	–	–	–
L-Histidine	–	–	d	–	–	+
L-Proline	–	+	+	–	–	+
L-Rhamnose	–	–	–	–	–	–
Sarcosine	–	–	–	–	–	–
Betaine	w+	–	–	–	–	–
Hippurate	–	–	–	–	–	–
n-Acetylglucosamine	–	+	+	–	–	–
D-Ribose	–	+	+	+	+	+
D-Glucose	–	+	+	–	–	+
D-Fructose	–	+	+	–	+	+
Maltose	–	+	+	–	–	+
Valerate	–	–	–	–	–	+

Table 13 continued:

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	no. 29	no. 55	no. 68
Heptanoate	–	–	–	–	–	–
DL-Lactate	–	+	–	–	–	+
DL-β-Hydroxybutyrate	–	–	–	–	–	–
Acetate	–	+	+	–	–	–
*iso*Valerate	–	–	–	–	–	+
*iso*Butyrate	–	–	–	–	–	–
Glycerol	–	+	+	–	+	–
cis-Aconitate	–	–	d	–	+	–
L-Threonine	–	+	+	–	+	–
D-Quinate	+	–	–	–	–	–
Benzoate	–	–	–	–	–	–
Hydroxymethylglutarate	–	N/A	N/A	–	–	–
Asparagine	–	+	N/A	–	–	+
Adenine	–	–	N/A	–	–	–
Xanthine	–	–	N/A	–	–	–
Caprate	–	+	–	–	–	–
Caprylate	–	–	–	–	–	–
Caproate	–	–	–	–	–	–
Ethanolamine	–	–	N/A	–	–	–
Glutarate	–	–	–	–	–	+
meso-Erythritol	–	–	–	–	–	–
Phenylacetate	–	–	–	–	–	–
Pelargonate	–	–	–	–	–	–

N/A = not available. d = 11-89% strains positive (Baumann *et al.* 1984).

Two of these tests were for reduction of nitrate to nitrite and growth at 4°C. The other 14 were carbon source utilization tests.

In agreement with the clustering in dendrogram 5, the comparisons derived from Table 13 showed that strain 29 was most similar to *V. cyclosites*, with 88% similarity. Differences were observed between the regional strain and this type

species in only a small number of tests, and none of these tests were for the functioning of major metabolic pathways, for example, the Voges-Proskauer test or the test for nitrate reduction.

V. ordalii was considered less similar to the regional strain because it differed from strain 29 in the nitrate reduction test [Table 13]. It also differed from strain 29 in a greater number of other traits than did *V. cyclosites*. The number of traits in which *V. marinus* differed from strain 29 was approximately twice the number in which *V. cyclosites* differed, *i.e.* 23 as opposed to 11.

Based on the preceding observations, strain 29 was identified as *V. cyclosites*. It should be noted that this identification was based only on this study's results for *V. cyclosites*, since no published description could be found.

The comparisons for strain 55 in Table 13 showed that strain 55 differed from *V. cyclosites* in 24 tests. Between these two strains, then, there was 74% similarity. Of the 24 tests in which differences were observed, two were growth temperature tests, for 4 and 35°C, and one was the test for nitrate reduction. The rest were carbon source utilization tests. Differences were observed between strain 55 and *V. marinus* in 22 tests, indicating 76% similarity. As well as the results of tests for the utilization of 19 carbon sources, there were differences in growth at 30°C, production of chitinase, and nitrate reduction. Strain 55 differed from *V. ordalii* in 18 tests, indicating 80% similarity. Of these tests, two were tests for growth at 4 and 30°C. The rest were carbon source utilization tests. The clustering in dendrogram 5

indicated that strain 55 was most similar to *V. marinus*. The comparisons in Table 13, however, showed that strain 55 was most similar to the published description of *V. ordalii*. This may be a reflection of discrepancies between this study's results for *V. ordalii*, used in the generation of dendrogram 5, and the published description of *V. ordalii*, used in Table 13. The published description of *V. ordalii* was considered most similar to strain 55 because it had the highest percentage of similar results, *i.e.* 80%, to the results of strain 55. Another observation supporting this decision was, of the three type species compared to strain 55, only *V. ordalii* agreed with the regional strain in the nitrate reduction test [Table 13]. None of the traits by which strain 55 and *V. ordalii* differed involved the function of a major metabolic pathway. Therefore, strain 55 was tentatively identified as *V. ordalii*.

In a detailed study of strain 68, Table 13 showed that strain 68 differed from *V. cyclosites* in 29 tests, indicating 68% similarity. Of these 29 tests, two were growth temperature tests, for 4 and 35°C, and one was the test for nitrate reduction. The rest were carbon source utilization tests. For strain 68 and *V. marinus*, 23 tests gave mismatches, indicating 75% similarity. Mismatches included growth at 30°C, production of chitinase, and nitrate reduction. The remainder of the 23 tests were for carbon source utilization. Strain 68 differed from *V. ordalii* in 22 tests, indicating 76% similarity. Two of these tests were for growth at 4 and 30°C, and the rest were carbon source utilization tests. As was observed for strain 55, dendrogram 5 showed strain 68 to be most similar to *V. marinus*. Again, however, comparison with the

published description of *V. ordalii* [Table 13] showed the regional strain to be most similar to *V. ordalii*. This was based on the observation that although strain 68 had very close similarity percentages, *i.e.* 75% and 76%, to *V. marinus* and *V. ordalii*, its result for nitrate reduction differed from that of *V. marinus*, but not from that of *V. ordalii* [Table 13]. The test for nitrate reduction reflects the function of a major metabolic pathway, and would be considered to distinguish one strain from another more definitely than would a difference in growth temperature results. Strain 68, then, was tentatively identified as *V. ordalii*. Like strain 55, this strain grew at 30 and 4°C, while *V. ordalii* didn't [Table 13]. Of the 20 carbon source utilization tests in which strain 68 differed from *V. ordalii*, strain 55 also differed, in the same way, in nine of these tests. The nine tests were: sucrose, *n*-acetylglucosamine, acetate, D-alanine, succinate, fumarate, D-galactose, L-tyrosine, and D-mannitol [Table 13]. In light of their tentative identifications as *V. ordalii*, strains 55 and 68 were noticeably similar.

Strains in group 2; clusters D to H of Figure 5:

Clusters D, E, F, and G of dendrogram 5 clustered sufficiently far away from any reference strains in this study, that they were thought to represent possible new species of *Vibrio*. The cluster attributes of these groups of regional strains are shown in Table 14.

Table 14. Table of test results representing entire clusters [clusters D, E, F, G, and H of Figure 5], based on character frequencies for each cluster. [Distinguishing characteristics are printed in boldface]

Test	Cluster D	Cluster E	Cluster F	Cluster G	Cluster H
Swarming	−*	−*	−*	−*	−*
Pigmentation	−*	−*	−*	−*	−*
Arginine Dihydrolase	−*	−*	−*	−*	−*
Oxidase	+	+*	+	+*	+*
NO_3-NO_2	−*	+*	d	d	+*
D-Glucose gas	−*	−*	−*	−*	−*
Voges-Proskauer	−*	−*	−*	−*	−*
growth at 40°C	−*	d	d	+	d
Sucrose	+	−*	d	d	d
Cellobiose	+	d	−	d	d
D-Gluconate	d	d	d	+*	d
γ-*Amino*butyrate	−	−*	−*	+*	d
Putrescine	−*	−*	−*	d	d
Chitinase	−*	d	−*	−*	d
growth at 4°C	+*	+	+*	+*	−*
growth at 20°C	+*	+*	+*	+*	+*
growth at 30°C	+*	d	+*	+*	+*
growth at 35°C	d	d	d	+	+*
Propionate	+*	+*	+*	+*	+*
DL-Malate	−	−*	d	d	d
L-Serine	+*	+*	+*	d	+*
L-Alanine	+*	+*	+*	+*	+
β-Alanine	−	−*	−	+	d
D-Alanine	d	+*	d	+*	+
L-Leucine	+	d	+*	+*	d
L-Tyrosine	+*	d	+	+*	d
Malonate	−	−*	d	+*	d
L-Glutamate	+*	+*	+*	+*	+*
L-Valine	−	−*	d	d	d
Succinate	+*	+*	+*	+*	+*
Fumarate	+*	+*	+*	+*	+*
L-Tartrate	−*	−*	−	−*	−
DL-Glycerate	−	d	d	+	d
Glycine	+	+*	d	+	d

Table 14 continued:

Test	Cluster D	Cluster E	Cluster F	Cluster G	Cluster H
DL-Aspartate	+	+*	+	d	+*
L-Ornithine	d	d	−*	d	+
L-Citrulline	+*	d	+*	d	d
D-Xylose	+	−	d	d	d
L-Arabinose	−	−*	d	d	d
D-Mannose	+*	+*	+*	d	d
D-Galactose	+*	+*	+*	d	d
D-Trehalose	d	+*	+*	d	+*
α-Lactose	+*	+*	+	d	−
D-Melibiose	+*	−	+*	d	d
D-Glucuronate	−	d	−	d	d
Salicin	−*	−*	+*	−	d
D-Galacturonate	−*	−*	−*	−	d
Citrate	+	+*	+*	+*	d
α-*Keto*glutarate	−*	−*	−*	−*	−*
Sodium Pyruvate	+*	+*	+*	+*	+*
Ethanol	−*	−*	+	+*	d
Propanol	−	−*	+	+*	d
D-Mannitol	+*	+*	+*	+*	+
D-Sorbitol	−*	−*	+	−	d
meso-Inositol	−*	−*	+*	d	d
p-Hydroxybenzoate	d	−*	+*	−	d
L-Histidine	−*	+*	+*	d	d
L-Proline	+	+*	+*	+*	+*
L-Rhamnose	−*	−*	+*	d	−
Sarcosine	−*	−*	+	d	−
Betaine	−*	−*	+*	+	d
Hippurate	−*	−*	d	−*	−
n-Acetylglucosamine	−	+*	+*	d	+*
D-Ribose	−	d	+	+	+
D-Glucose	+*	-*	+*	+*	d
D-Fructose	+*	−*	+*	+*	d
Maltose	+*	+	+*	+*	+
Valerate	+	−*	d	+*	d
Heptanoate	+	−*	d	+*	+
DL-Lactate	−	+*	d	+*	+*
DL-β-Hydroxybutyrate	−	−*	−	+*	d
Acetate	+*	+*	+*	+*	d

Table 14 continued:

Test	Cluster D	Cluster E	Cluster F	Cluster G	Cluster H
*iso*Valerate	−*	−*	−	+*	d
*iso*Butyrate	−*	−*	−	−	d
Glycerol	d	+*	d	+	+*
cis-Aconitate	d	+*	+	+*	−
L-Threonine	+*	+*	+*	d	d
D-Quinate	−	−*	−	−*	d
Benzoate	−	−*	−	−*	−
Hydroxymethylglutarate	−	−*	−	−*	−*
Asparagine	d	+*	d	+*	+
Adenine	−*	−	−*	−*	−*
Xanthine	−*	−*	−	−*	−*
Caprate	d	d	+*	+*	+
Caprylate	d	−*	−*	d	d
Caproate	−*	−*	−*	−*	d
Ethanolamine	−*	−*	−	−	−*
Glutarate	−*	−*	d	d	d
meso-Erythritol	−*	−*	−	−*	−
Phenylacetate	−*	−*	d	−*	−*
Pelargonate	d	−	d	+*	d

An asterisk [*] beside a positive or negative result means that all strains in that cluster gave a positive or negative result, respectively, for the test. In other words, the frequency in these cases was 100%

d = 16-84% strains positive.

Cluster D [Fig. 5] consisted of 14 regional strains from alga, all from the D1 sub-collection. The strains shared a number of characters, as was indicated by the few d results [Table 14]. All strains in the cluster were in complete agreement in 30% of the tests, as indicated by the asterisks. Most strains were in agreement in 57% of the

tests. The results for cluster D had more positive test results than, for example, cluster A had shown. This indicated that strains from alga were more nutritionally versatile than strains from scallop. This cluster of strains from alga may represent a new *Vibrio* species, or a new biovariety.

Cluster E [Fig. 5] was formed by nine regional strains from alga. Five were from the D2 sub-collection and four from the D3 sub-collection. Cluster E strains shared many properties, with all strains giving the same result in 78% of the tests [Table 14]. There were relatively few d results. The strains of cluster E were not similar to any existing type species used in this study, so they may represent a new *Vibrio* species, or biovariety.

Cluster F [Fig. 5] was comprised of six regional strains from alga and one from scallop. Of the strains from alga, five were D1 strains and one was a D2 strain. The strains of cluster F showed matches for 50% of the tests. They possibly represent a new *Vibrio* species, or biovariety. Baumann *et al.* (1984) stated that several species of *Vibrio* are able to utilize the aromatic compounds benzoate, *p*-hydroxybenzoate, or quinate. All the strains in cluster F were able to utilize *p*-hydroxybenzoate as sole source of carbon and energy [Table 14]. This was the only example in this study where all the strains in a cluster were able to utilize one of the above three compounds cited by Baumann *et al.* (1984).

Cluster G [Fig. 5] consisted of five regional strains from alga and three from scallop. From alga, three were D1 strains and two were D2 strains. Cluster G was

much like cluster F, because of the combination, in both clusters, of regional strains from alga and scallop. Matched results for strains in cluster G occurred in 55% of the tests [Table 14]. They may represent a new *Vibrio* species, or biovariety, isolated from two very different sources.

Cluster H [Fig. 5] consisted entirely of reference cultures, clustering together rather than with any regional strains. Matched results, for all strains in this cluster, occurred in only 32% of the tests, and there were many d results. This was probably because it was composed entirely of type species, where differences would be expected.

Distinguishing characteristics of Clusters D, E, F, and G were observed, and are printed in boldface type in Table 14.

Determinative Tests that Helped to Distinguish Between Clusters:

Once the tentative identifications of the regional strains had been made, a study was done to determine if all the tests used from Baumann *et al.* (1984) and West *et al.* (1986) contributed to these identifications. It was speculated that some tests would be more important determinative characters than others. The percentage of strains in each cluster that were positive for each test, *i.e.* the percentage frequencies of positive characters, had been calculated [Appendix G]. These percentage frequencies showed which tests gave uniform results among all clusters, and which tests gave a result in one cluster different from those of all other clusters.

The former tests, then, were those of little determinative value in the clustering process, while the latter tests distinguished individual clusters.

Because the tentative identifications in this study were initially based on the separation of study strains into groups of arginine dihydrolase-positive strains and arginine dihydrolase-negative strains, the determination of key tests in this study was done separately for these two groups. The percentage frequencies of positive characters for the arginine dihydrolase-positive strains in clusters A to C of dendrogram 4 [Fig. 4], were compiled and are given in Appendix G, Table 1. Those for the arginine dihydrolase-negative strains in clusters A to H of dendrogram 5 [Fig. 5], were compiled and are given in Appendix G, Table 2. Any character which occurred with approximately the same frequency in all clusters did not contribute to the distinction of clusters. A set of tests, which did not contribute to distinction of clusters for either of the arginine dihydrolase-negative or positive strains, was determined. These are shown in Table 15, derived from Appendix G, Tables 1 and 2.

When considering just the arginine dihydrolase-positive strains [Fig. 4], the arginine dihydrolase test did not define any clusters because it was common to all clusters. In addition to the tests in Table 15, there was another set of tests found to be of little use in distinguishing between arginine dihydrolase-positive strains. This set is shown in Table 16, derived from Appendix G, Table 1. The combined tests in Tables 15 and 16 were of little determinative value for arginine dihydrolase-positive strains.

Table 15. Tests which were of little determinative value for all strains in this study

Swarming	Pigmentation	Oxidase
D-Glucose gas	Voges-Proskauer	growth at 20°C
Propionate	L-Glutamate	Succinate
Fumarate	L-Tartrate	D-Galacturonate
α-*Keto*glutarate	Sodium pyruvate	Hippurate
*iso*Butyrate	D-Quinate	Benzoate
Hydroxymethylglutarate	Adenine	Xanthine
Ethanolamine	Glutarate	*meso*-Erythritol
Phenylacetate		

Table 16. Tests which were of little determinative value for arginine dihydrolase-positive strains

L-Alanine	β-Alanine	Malonate
DL-Aspartate	D-Melibiose	D-Glucuronate
Citrate	*meso*-Inositol	*p*-Hydroxybenzoate
L-Histidine	L-Proline	L-Rhamnose
Sarcosine	Betaine	*n*-Acetylglucosamine

Characters which were of value in distinguishing clusters were those possessed by at least 80% of the strains in a cluster. As well, the percentage frequency, or frequency of occurrence, of the character in a cluster had to be at least twice as large as its percentage frequency in all the other clusters of the dendrogram. That is, the binary frequencies ratio (Hollohan 1982) of the character in the cluster it distinguished had to be at least 2.0.

A detailed study of Appendix G, Table 1, based on the criteria above, indicated which tests did distinguish single clusters from all others in dendrogram 4. These tests, and the clusters they distinguished, are presented in Table 17. The set of tests shown in Table 17 was sufficient to distinguish all three clusters in dendrogram 4. The remainder of the 91 tests from Baumann *et al.* (1984) and West *et al.* (1986) were not as useful determinative characters for the arginine dihydrolase-positive strains. The information offered by all tests, however, was valuable in the comparisons of the regional strains of dendrogram 4 to the type species with which they clustered.

When considering just the arginine dihydrolase-negative strains of dendrogram 5, the arginine dihydrolase test again did not define clusters because it was common to all of them. Nor did the tests for growth at 30°C and utilization of putrescine. These tests, in addition to those in Table 15, were the tests least useful as determinative tests for arginine dihydrolase-negative strains.

Table 17. Determinative tests that were most useful to define clusters in dendrogram 4 [Fig. 4]

Tests	Cluster distinguished by these characters
D-Galactose, α-Lactose, *cis*-Aconitate	A
NO_3-NO_2, L-Serine, Glycine	B
Sucrose, Cellobiose, D-Gluconate, Putrescine, growth at 35°C, DL-Malate, L-Ornithine, D-Fructose, Heptanoate, Caprylate	C

The tests which did distinguish arginine dihydrolase-negative strains were determined, based on the criteria of Hollohan (1982). These tests, and the clusters they distinguished, are presented in Table 18, derived from Appendix G, Table 2. As shown in Table 18, not all the clusters of dendrogram 5 were separated by this set of tests. It was found, from data in Appendix G, Table 2, that several other tests distinguished two clusters from all others in dendrogram 5.

These were: DL-glycerate, separating clusters B and D from all others; D-melibiose, separating clusters D and F from all others; betaine, separating clusters F and G from all others; and *iso*valerate, separating clusters C and G from all others. These tests, in combination with those in Table 18, distinguished all clusters in dendrogram 5 except for clusters A and E.

Table 18. Determinative tests that were most useful to define clusters in dendrogram 5 [Fig. 5]

Tests	Cluster distinguished by these characters
Maltose, Caprate	B
Salicin, D-Sorbitol, *p*-Hydroxybenzoate, L-Rhamnose	F
γ-*Amino*butyrate, β-Alanine, Malonate	G
growth at 4°C	H

It was concluded that for identification of the arginine dihydrolase-negative strains in this study, all the 91 tests from Baumann *et al.* (1984) and West *et al.* (1986) were necessary, except for growth at 30°C, utilization of putrescine, and the tests in Table 15. Although these tests were of little value for differentiating between the clusters of dendrogram 5, the information from all tests was valuable when comparing the regional strains to the type species with which they clustered. In dendrogram 5, all tests could help to characterize those clusters thought to represent new *Vibrio* species.

DISCUSSION

A main objective of this study was to use numerical analysis to determine similarities between bacteria that grow best under temperate conditions and those that are adapted to grow at colder temperatures, such as 4°C. The strains studied were type strains and reference cultures that represented *Vibrio* from a variety of sources, and sets of *Vibrio* isolated from the coastal waters of Newfoundland. The latter, which were called regional strains, were identified as *Vibrio* in earlier studies (Hollohan 1982 and Hollohan *et al.* 1986). The earlier studies emphasized bacterial succession, mostly at the generic level. The determination of moles % guanine plus cytosine was a part of the earlier studies. The study presented here was an extension of the earlier studies. The tests conformed more closely to those used by Baumann *et al.* (1984), for *Vibrio,* in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984) and a much larger number of type or reference cultures was used. These represented a number of species, many of which had not been described at the time that Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984) was published or the study of Hollohan *et al.* (1986) was undertaken. Also, in many instances, species descriptions published after the most recent Bergey's Manual were not as extensive as the descriptions for species given in Bergey's Manual. Therefore, there was a need to obtain some of this information experimentally. Other questions, such as optimal incubation temperature and times, remained to be investigated.

Another area of interest was the effect of the interpretation of weak positive results as either positive or negative.

Testing for Characteristics:

Bacterial strains, maintained in subculture and put through several passages of media, may lose or alter some of their original properties (Bryant *et al.* 1986). The maintenance of a strain by subculture can result in a decrease in its vigour and the loss of some characteristics. This can be a problem when dealing with large culture collections. It can be minimized over time, but not avoided. The strains used in this study were preserved under sterile mineral oil. This decreased the need for frequent subculture, but did not eliminate the possibility of strain variation or changes in vigour. Considering that some new tests were introduced in the present study, and a number of the reference cultures had not been studied previously in this laboratory, it was decided to repeat as many tests as practical to obtain the new data matrix. An exception was the test for luminescence. Results from earlier studies were negative for all regional strains. These had been tested extensively and it was decided that further tests would not alter the outcome (J. Gow, personal communication). Luminescence was not included as a test in the data matrix but it was accepted that the regional strains were negative for this character.

Choice of Incubation Temperature:

Baumann *et al.* (1984), in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984) stated that incubation temperatures of 25 and 30°C are adequate for most *Vibrio* species, and 15°C is recommended for a few. Also, they noted that each grew at 20°C. Gow and Mills (1984) showed that most bacteria from this region did not grow well at temperatures above 20°C, at least when first isolated. In the study presented here, all strains could grow at 20°C, but not all strains could grow at the other temperatures that were tested. An incubation temperature of 25°C was not tested because it was not listed as a test temperature in the determinative tables, for *Vibrio*, in Bergey's Manual of Systematic Bacteriology, Vol.1 (Krieg and Holt 1984). Also, this temperature was known to be above the limits for the growth of some psychrophilic *Vibrio*, such as *V. marinus* and *V. salmonicida* (Baumann *et al.* 1984; Egidius *et al.* 1986). Based on these observations, 20°C was chosen as the routine incubation temperature for all strains. *V. marinus* and *V. salmonicida* did not grow well in this study. Even 20°C may have been too high for their optimum growth. This was probably not the only reason for poor growth. Attempts to get good growth, even at lower temperatures, did not always meet with success.

Choice of Incubation Period for Growth on Organic Substrates as Sole Sources of Carbon and Energy:

The majority of *Vibrio* species that have been studied are mesophiles and tests for growth on organic compounds, as sole sources of carbon and energy, are routinely incubated for six days at 25°C (Baumann *et al.* 1984). There are exceptions. Schiewe *et al.* (1981) kept biochemical tests under observation for 14 days when they studied *V. ordalii*. Gow and Mills (1984) showed that strains from this region grew slowly, even at 20°C, when first isolated. One of the aims of this study was to determine if incubating carbon source utilization tests for six days, at 20°C, was adequate for a systematic study of cold-adapted strains. The tests are important because *Vibrio* classification is based, to a considerable extent, on nutritional characters. It was found that growth on some compounds required as long as three weeks to develop. This was observed for both regional and reference cultures. Three weeks was recommended as the incubation period.

Treatment of Weak Positive Results:

For many of the tests, results were recorded as negative [-], weak positive [w+], or positive [+]. Results that were read as weak positive were those that were difficult to interpret because growth was sparse or otherwise weaker than observed for the positive controls. For the carbon source utilization tests this indicated some use of the substrate, but it could not be considered equal to a display of good growth

which would be clearly read as positive. These are the tests for which personal interpretation would have the most effect on scoring a positive or negative result. When compiling data for their tables of determinative characteristics of the genus *Vibrio*, Baumann *et al.* (1984) noted that the inclusion of tests supporting relatively light growth was unavoidable. Their examples included the carbon sources such as propionate, valerate, heptanoate, ethanol, and L-glutamate. Examples of substrates that gave weak positive growth, in the present study, were L-leucine, L-ornithine, asparagine, and xanthine.

Dendrograms were generated for which weak positive data was treated as positive, weak positive, or negative. From the analysis of the three data states, and no matter which way the weak positive results were scored to accommodate unweighted cluster analysis, the regional strains, for the most part, tended to cluster among themselves, and away from the reference strains. This supported one of the hypotheses proposed for this study. That is, based on phenetic characters, some clusters of regional strains would be different from the type and reference cultures.

Determinative Characters; Selection of Limits for Assigning Positive and Negative Values to Characters That Describe Clusters of Strains:

For clusters of strains that were of interest the frequencies for positive and negative results, for each character in the cluster, were obtained. The frequencies, expressed as a percentage, gave a value by which a decision could be made that all

strains possessed the character, or that all strains did not possess the character, or that the strains may, or may not, possess the character. By this process attributes of strains within a cluster were determined as +, -, or d, and these values could be compared with the corresponding attributes of strains in other clusters or with type cultures.

The values that would define the upper and lower limits of positive and negative results had to be decided. In Bergey's Manual of Systematic Bacteriology, Vol. 1 (Krieg and Holt 1984), Baumann *et al.* (1984) called all strains positive if more than 89% of the strains in the cluster possessed the character. If the percentage of positive results for any character was less than 11% then all strains in the cluster were designated negative for that test. If 11-89% of the strains in the cluster were positive then that character was given the designation d. In the present study, a d value was assigned to a character if 16-84% of the strains in a cluster were positive. These limits, which were somewhat less restrictive than those used by Baumann *et al.* (1984), were chosen because several of the clusters in this study contained small numbers of strains. For example, if a cluster had seven strains and the 90% limit of Baumann *et al.* (1984) was used, only those tests that elicited 100% positive or negative results in the cluster would be called +, or -, respectively. Using the scheme of the present study, if six of the seven strains were positive for a test, this would be translated into a frequency of 85% positive and scored +. According to the limit of Baumann *et al.* (1984), the same test result for the cluster would be designated d. In

other studies an 85% limit has been used, for example, by Cowan (1974b). A lower limit was employed by West *et al.* (1985) and Smith *et al.* (1991), who used 80%. The 85% limit was selected for this study so that it could be said that, if almost all strains gave a matching result for a test, the result would be representative of the entire cluster of strains. Sufficient data is presented in the thesis that other limits could be calculated if other investigators were interested in doing this.

Identification of Arginine Dihydrolase-Positive and Arginine Dihydrolase-Negative Strains:

In several studies of *Vibrio*, once it was determined whether the study strains were positive or negative for the arginine dihydrolase test, the strains were then compared with known *Vibrio* species having the same arginine dihydrolase result. Studies which have employed this method include that of Tison and Seidler (1983), in their study of *V. aestuarianus*, and Pujalte and Garay (1986), in their study of *V. mediterranei*. It is important because it is a system whereby ATP can be generated. Some bacteria are able to generate usable energy, in the form of ATP, by the degradation of arginine via the arginine dihydrolase system (Moat 1979). This pathway is thought to provide the energy needed for growth and motility under anaerobic conditions, and may also be induced under conditions of extreme nutrient limitation such as carbon source or phosphate depletion (Phillips 1986). It might be concluded, then, that the arginine dihydrolase test serves to distinguish *Vibrio* species

adapted to survival in anaerobic or adverse environments.

Baumann *et al.* (1984) stated that the presence of an arginine dihydrolase system is diagnostic for a number of species of *Vibrio*. It is generally considered unlikely that an arginine dihydrolase-negative bacterial strain, for example, would be closely related to another strain, even in the same genus, that is arginine dihydrolase-positive. In the study presented here, the practice of studying arginine dihydrolase-positive strains separately from arginine dihydrolase-negative strains was followed.

Identification of the Arginine Dihydrolase-Positive Strains:

The study of the arginine dihydrolase-positive regional strains showed that these were all from alga and all but one were identified as *V. splendidus* biovar I. The *V. splendidus* biovar I strains were found in two closely related clusters. There were two clusters because of some differences in test results between strains in the two clusters. These differences will be discussed later. However, the test results for the majority of strains, identified as *V. splendidus* biovar I, were in general agreement with the published description. The traits for which changes to the species description of *V. splendidus* biovar I can be proposed are given in table form [Table 19]. These changes are based on properties of the regional strains which were different from the published description of *V. splendidus* biovar I (Baumann *et al.* 1984). The changes consist of denoting *V. splendidus* biovar I results for some tests as d, rather than positive or negative, to accommodate the results of the regional strains.

Table 19. Proposed changes to the description of *V. splendidus* biovar I to include properties of the regional strains

Test	Trait in current description of *V. splendidus* biovar I	Change proposed to include regional strains
Growth at 30°C, production of Chitinase, Cellobiose, Propionate, DL-Malate, Glycine, L-Citrulline, D-Glucuronate, α-*Keto*glutarate, D-Glucose, D-Fructose, Heptanoate	+	d
L-Ornithine, α-Lactose, D-Melibiose, Asparagine	−	d

d = 16-84% strains positive.

Most of the regional strains of *V. splendidus* biovar I may be distinguished from previously described strains of *V. splendidus* biovar I by the following characters. They are able to utilize α-lactose and asparagine as sole sources of carbon and energy, and not able to utilize DL-malate, α-*keto*glutarate, or heptanoate. The four strains of *V. splendidus* biovar I described by Baumann *et al.* (1984) were luminescent. The regional strains from the study reported here were negative for this character. West *et al.* (1986) studied eight strains of *V. splendidus* including the type strain for biovar I [ATCC 33125], also used in the present study. These authors reported all strains negative for luminescence. It was decided that non-luminescence in regional strains should not detract from their identification as *V. splendidus* biovar I.

V. splendidus biovar I and the majority of the arginine dihydrolase-positive strains displayed a high degree of dissimilarity to other arginine dihydrolase-positive strains, most of which were type cultures. It was of interest to note that some strains of *V. splendidus* biovar I are known to grow at 4°C while most of the other type cultures do not (Baumann *et al.* 1984).

Tests that define genotypic similarity, such as restriction endonuclease assays, could be carried out to confirm that the majority of the arginine dihydrolase-positive regional strains were *V. splendidus* biovar I. Also, *V. splendidus* biovar I is known to produce amylase, gelatinase, and lipase (Baumann *et al.* 1984), but not elastase or alginase (West *et al.* 1986). Because the present study did not include many tests for production of exoenzymes, tests for the enzymes mentioned above might also be carried out on these regional strains.

Two regional arginine dihydrolase-positive strains, that were identified as *V. splendidus* biovar I, were found in a small cluster along with the type species of *V. splendidus* included in the study. Differences in a small number of characters resulted in the formation of this additional cluster. This can result from near-optimal rather than optimal merging of clusters by Ward's method (Romesburg 1984). The most significant difference between the three strains, in the smaller of the two clusters, and the remaining regional strains was that the former did not reduce nitrate. According to Baumann *et al.* (1984) the type strain should reduce nitrate. A further more intensive study of this character may show all strains to be positive for nitrate

reduction, or may confirm the results shown here. Additional testing of this character is recommended if these strains are studied further. The two regional strains in the smaller cluster were not as nutritionally versatile as the majority of the other arginine dihydrolase-positive strains. They were unique among the regional strains in this study by the following combination of traits: they utilized DL-glycerate as a sole source of carbon and energy, and did not utilize L-serine, caprate, or D-gluconate.

One arginine dihydrolase-positive strain clustered with the majority of the type strains. This strain was the only arginine dihydrolase-positive regional strain that utilized D-xylose as sole source of carbon and energy, a trait considered unusual in the genus *Vibrio* (Guerinot *et al.* 1982). This trait indicated similarity of the regional strain to *V. diazotrophicus*, a species known to utilize D-xylose (Guerinot *et al.* 1982). In this study, *V. aestuarianus* also utilized D-xylose, but its published result, unlike that for *V. diazotrophicus*, was not available. Because there was some relationship, *i.e.* 73% similarity, between this regional strain and *V. diazotrophicus*, the performance of one additional test would help to clarify this relationship. *V. diazotrophicus* is a nitrogen-fixing bacterium (Guerinot *et al.* 1982). It could be determined with more certainty if the strain is *V. diazotrophicus* by testing it for nitrogenase activity. In addition it could be tested for production of amylase and phosphatase, for casein hydrolysis and especially the production of gelatinase and deoxyribonuclease. *V. diazotrophicus* is described as positive for the former two enzymes, and negative for the latter three (Guerinot *et al.* 1982). *V. aestuarianus*, in contrast, is known to

produce gelatinase and deoxyribonuclease (Tison and Seidler 1983). DNA studies and perhaps carbon source utilization tests on even more substrates may be necessary to determine if this regional strain can be identified as either of these *Vibrio* species. *V. diazotrophicus* and *V. aestuarianus* are both known to grow at 4°C.

Identification of the Arginine Dihydrolase-Negative Strains:

The numerical analysis of the arginine dihydrolase-negative strains resulted in the formation of two groups of clusters. Considered for further study were three clusters in one group and five clusters in a second group. Strains in the first group were compared to three reference cultures that clustered among these groups. These were *V. marinus*, *V. cyclosites*, and *V. ordalii*. The majority of strains from this group were most similar to *V. marinus*. Although *V. marinus* and *V. ordalii* are currently recognized species of *Vibrio* (Baumann *et al.* 1984; Schiewe *et al.* 1981), the status of *V. cyclosites* is less certain. It was included as a reference culture in this study because it is listed as a *Vibrio* species in the 1989 edition of the American Type Culture Collection catalogue. A search of the Approved Lists of Bacterial Names (IJSB 30: 225-420, 1980) and issues of the International Journal of Systematic Bacteriology published since January 1980 was undertaken and no evidence was found that this is a validly published type species. However, its inclusion was not seen as detracting from this study. Should valid publication occur at a later date then data about the relationship of this organism to the regional strains will be available.

One strain, isolated from *A. esculenta*, was similar to the *V. cyclosites* reference culture used in this study. The regional strain was considered atypical because it grew at 4°C and not at 30 or 35°C. The atypical growth temperature results of this regional strain would be most likely because of its cold-ocean source of isolation. The type strain of *V. cyclosites* was isolated from soil (ATCC Catalogue of Bacteria and Bacteriophages 1989). Testing of strain 29 for decomposition of phenol and *m*-cresol would also help to determine if this strain is indeed *V. cyclosites*, because strains of this species decompose these substances.

Most strains in group 1 were able to reduce nitrate. This is a property of *V. marinus* rather than *V. ordalii*. It is an important test in the determinative table of Baumann *et al.* (1984). This would support the identification of the strains as *V. marinus*. Also, *V. marinus* is known to grow at 4°C. To further verify the identification of these strains as *V. marinus*, further studies can be recommended. These include restriction endonuclease assays. Also, because *V. marinus* is known to produce lactate dehydrogenase (ATCC Catalogue of Bacteria and Bacteriophages 1989), gelatinase, and lipase, but not amylase or alginase (Baumann *et al.* 1984), the tests for the production of these enzymes could be done.

The published description of *V. marinus* is based on the characteristics of only one strain (Baumann *et al.* 1984). The study presented here contained 19 strains that were identified as *V. marinus*. While these strains were most similar to this species, they did differ completely from the published description in certain tests. All the

regional strains were negative for utilization of D-gluconate, DL-malate, and α-*keto*glutarate. They were positive for growth at 30°C, and utilization of sodium pyruvate and propionate. In addition, regional strains in one cluster [cluster A of Fig. 5] were unique by testing negative for utilization of D-alanine, D-galactose, L-proline, and glycerol. Regional strains in another cluster [cluster C of Fig. 5] were unique in testing negative for production of chitinase, utilization of D-ribose and D-fructose, and positive for utilization of DL-glycerate, valerate, and *iso*valerate. All these attributes of the regional strains were opposite to those of *V. marinus*.

The regional strains that were identified as *V. marinus* were all isolated from *P. magellanicus*, the giant scallop. A characteristic of *V. marinus* is that it can use relatively few carbon compounds as sole sources of carbon and energy (Baumann *et al.* 1984). If the regional bacteria were commensals, the scallop itself may have provided a favourable environment for these bacteria.

The published test results of *V. marinus* were used throughout this study. There was difficulty in maintaining good growth of this reference strain, and therefore the full complement of 91 tests was not carried out. Cultures were obtained from the ATCC several times throughout the study but, after repeated failure to maintain adequate growth, it was decided to proceed with numerical analysis using published results. The published description is based on properties of one strain (Baumann *et al.* 1984).

One strain from alga and one from scallop were identified as *V. ordalii*. Here

there was general agreement between the results obtained for the type culture included in the study and the published description of *V. ordalii* (Baumann *et al.* 1984). Reassignment as *V. ordalii* was proposed by Schiewe *et al.* (1981) for strains previously named *V. anguillarum* biovar II. Differences between the regional strains and the description of *V. ordalii* are summarized in Table 20. Unlike the regional strains, *V. ordalii* is not known to grow at 4 and 30°C. Also, *V. ordalii* has been shown to be negative for starch hydrolysis, lipase activity, and the ONPG test (Schiewe *et al.* 1981). These characters were not determined in this study.

Table 20. Proposed changes to the current description of *V. ordalii* to include strain no.'s 55 and 68

Test	Trait in current description of *V. ordalii*	Proposed change to include strain no.'s 55 & 68
Growth at 4°C, Growth at 30°C, D-Alanine, D-Gluconate, Succinate, Fumarate, D-Galactose, Cellobiose, L-Tyrosine, γ-*Amino*butyrate, D-Trehalose, D-Mannitol, DL-Lactate, Propionate, L-Alanine, L-Leucine, D-Mannose, Valerate, *iso*Valerate, Glutarate	−	d
Sucrose, L-Proline, D-Glucose, *n*-Acetylglucosamine, Maltose, Acetate, Citrate, Glycerol, L-Threonine	+	d

d = 16-84% strains positive.

V. ordalii is a causative agent of vibriosis, a serious infectious disease in marine fish, and all *V. ordalii* cultures have been isolated from the tissues of diseased marine fish (Schiewe *et al.* 1981). As an example, the ATCC strain of this species, used in this study, was isolated from the kidney of a Coho salmon, *Onchorhynchus kisutch* (ATCC Catalogue of Bacteria and Bacteriophages 1989). If the two regional strains are ultimately identified as *V. ordalii*, this would be two new sources of isolation for this species. One strain was isolated from fronds of brown alga, and the other from the giant scallop. These may be reservoirs of this organism. This could have implications in aquaculture. Even if the strains are not found to be virulent, other strains, upon isolation from the same sources, may be similar enough to be readily confused with virulent strains. Further studies would be required to better characterize virulent and avirulent strains.

The arginine dihydrolase negative-strains clustered into two main groups. The first group, identified as mostly *V. marinus*, was described above. The second group consisted of four clusters of regional strains and a cluster of type cultures. Interestingly, growth at 4°C was a character that could be used to distinguish between the four clusters of regional strains and the cluster of type cultures. The cold-adapted strains grew at this temperature while the type cultures did not. The regional strains in the four clusters were not identified. However, some comments can be made about their properties. In addition, some comparisons with literature descriptions of *V. salmonicida* (Egidius *et al.* 1986) and *V. navarrensis* (Urdaci *et al.*

1991) are made. In the present study, so many negative results were obtained for *V. salmonicida* that there was a possibility that the organism was not growing well enough to give good results. The results obtained were not used. *V. navarrensis* was described so recently that the strain was not included for test results. The current literature descriptions of these organisms contained much fewer characters than were used in the data base of the present study.

One cluster [cluster D of Fig. 5] was noticeable by its distance from any clusters containing reference strains. All the strains were regional strains from the first alga collection. They utilized D-xylose as sole source of carbon and energy. Guerinot *et al.* (1982) stated that the ability to utilize D-xylose as a sole source of carbon and energy is unusual in the genus *Vibrio*. Because the cluster D strains had this ability, and were not similar to species described previously, it was thought likely that they may represent a new species. In addition, based on just the utilization of D-xylose, these strains did not appear to be either *V. salmonicida* or *V. navarrensis*. D-xylose is not utilized by *V. salmonicida* (Egidius *et al.* 1986) or *V. navarrensis* (Urdaci *et al.* 1991). There were some other regional strains which also utilized D-xylose but these did not all cluster together, as did the strains in cluster D.

A second cluster [cluster E of Fig. 5] consisted of regional strains from the second and third alga collections. These were thought to represent a new *Vibrio* species because they clustered distantly from all reference strains. This cluster was distinguishable from all other strains in the study by the utilization of glycine, but not

D-glucose, as sole source of carbon and energy. These strains were different from *V. salmonicida* and *V. navarrensis* because both type species are able to utilize D-glucose (Egidius *et al.* 1986; Urdaci *et al.* 1991). Glycine utilization by the two type species was not available in the literature.

The third cluster [cluster F of Fig. 5] consisted of strains from the first and second alga collections, and one strain from scallop. The possible new species represented by this cluster may be distinguishable by the following traits, possessed by all strains in the cluster. They utilized salicin, *meso*-inositol, *p*-hydroxybenzoate, L-rhamnose, and betaine as sole sources of carbon and energy. They were unlike *V. salmonicida* because of its inability to utilize salicin, inositol, and rhamnose (Egidius *et al.* 1986), and unlike *V. navarrensis* because of its inability to utilize *myo*-inositol, rhamnose, *p*-hydroxybenzoate, and betaine (Urdaci *et al.* 1991).

The fourth cluster [cluster G of Fig. 5] consisted of strains from the first and second alga collections, and three strains from scallop. An interesting observation here was that the strains from scallop remained separate from the strains from alga by forming subclusters. For the present study, however, all eight strains were considered as one cluster. The possible new species represented by this cluster may be distinguished by the following traits. These strains utilized malonate, ethanol, propanol, heptanoate, DL-β-hydroxybutyrate, pelargonate, D-gluconate, and γ-*amino*butyrate as sole sources of carbon and energy. All of them hydrolyzed laminarin [Appendix B]. They were unlike *V. navarrensis* based on the type species'

inability to utilize malonate, DL-β-hydroxybutyrate, and γ-*amino*butyrate (Urdaci *et al.* 1991). *V. salmonicida* could not be compared to the cluster G strains because its description did not include results for most of these distinguishing characteristics (Egidius *et al.* 1986).

General Conclusion:

The purpose of this study was to determine similarities and differences between bacteria that grow naturally at 4°C and those that do not. The strains studied were *Vibrio* type and reference cultures, and strains isolated from a seasonally-cold ocean. A significant finding in this study was that most regional strains, that could be identified, belonged to species that are known to grow at 4°C. These were 19 strains of *V. splendidus* biovar I from alga and 19 strains of *V. marinus* from scallop. Three strains were identified as species that are not known to grow at 4°C. These were strains of *V. ordalii* and *V. cyclosites*. A fourth strain may have been *V. diazotrophicus* or *V. aestuarianus*, both of which can grow at 4°C. Four clusters of regional strains were not identified. Associated with these four clusters was a fifth cluster containing type cultures that are not known to grow at 4°C. This was an important determinative character that distinguished between these type cultures and the unidentified regional strains. This study has shown that some, but not all, cold-ocean *Vibrio* can be described by an existing classification scheme. Additional studies will be required to further describe the strains that were not identified.

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APPENDIX A

Appendix A. Regional strains included in this study; original numbers and dates of collection, and corresponding numbers in this study

Original no.	Collection Date	no. in this study
Collected from *A. esculenta*:		
D1-1	June 13, 1979	1
D1-2	"	47
D1-3	"	2
D1-4	"	3
D1-5	"	4
D1-6	"	5
D1-7	"	6
D1-8	"	7
D1-9	"	8
D1-10	"	48
D1-11	"	9
D1-14	"	10
D1-15	"	11
D1-16	"	12
D1-18	"	49
D1-19	"	13
D1-22	"	14
D1-23	"	50
D1-28	"	51
D1-30	"	15
D1-39	"	52
D1-40	"	16
D2-8	August 15, 1979	17
D2-11	"	18
D2-12	"	19
D2-14	"	53
D2-17	"	54
D2-18	"	20
D2-19	"	21

Appendix A continued:

Original no.	Collection Date	no. in this study
D2-23	"	22
D2-27	"	55
D2-30	"	56
D2-33	"	57
D2-35	"	23
D2-37	"	58
D2-38	"	59
D2-39	"	24
D2-40	"	25
D2-42	"	26
D2-43	"	27
D2-44	"	60
D3-4	September 20, 1979	28
D3-6	"	61
D3-7	"	62
D3-8	"	29
D3-9	"	30
D3-11	"	31
D3-12	"	63
D3-14	"	32
D3-15	"	33
D3-16	"	34
D3-17	"	35
D3-24	"	64
D3-25	"	36
D3-29	"	37
D3-30	"	65
Collected from *P. magellanicus*:		
P-2	May 25, 1977	66
P-7	"	67
P-10	"	68
P-12	"	69
P-14	"	70
P-17	"	71
P-20	"	38

Appendix A continued:

Original no.	Collection Date	no. in this study
P-26	"	72
P-34	"	39
P-36	"	40
P-40	"	73
P-50	"	41
P-53	"	42
P-57	"	74
P-66	"	75
P-72	"	43
P-73	"	44
P-75	"	45
P-77	"	76
P-78	"	77
P-79	"	46
P-89	"	78
P-92	"	79
P-95	"	80

APPENDIX B

Appendix B. Hydrolysis of Laminarin

Strain no.	Result	Strain no.	Result	Strain no.	Result
1	-	29	-	57	-
2	+	30	-	58	-
3	-	31	-	59	-
4	+	32	-	60	-
5	+	33	-	61	+
6	-	34	-	62	+
7	+	35	-	63	-
8	+	36	-	64	-
9	-	37	-	65	-
10	-	38	-	66	-
11	-	39	-	67	-
12	+	40	-	68	-
13	+	41	-	69	-
14	+	42	-	70	-
15	+	43	-	71	-
16	·	44	-	72	-
17	+	45	-	73	-
18	+	46	-	74	-
19	-	47	+	75	-
20	-	48	-	76	-
21	-	49	-	77	-
22	-	50	-	78	-
23	-	51	-	79	-
24	-	52	-	80	-
25	-	53	-		
26	-	54	-		
27	-	55	-		
28	-	56	-		

APPENDIX C

List for Appendix C, Table 1.

List of tests which made up the boldface tests of the scheme of Baumann *et al.* (1984) [Test no.'s lined up across top of Appendix C, Table 1]

Test no.	Name of Test
1.	Swarming
2.	Pigmentation
3.	Arginine Dihydrolase
4.	Oxidase
5.	Reduction of Nitrate to Nitrite
6.	Production of Gas from the Fermentation of D-Glucose
7.	Voges-Proskauer test
8.	Growth at 40°C

Carbon Source Utilization tests:

9.	Sucrose
10.	Cellobiose
11.	D-Gluconate
12.	γ-*Amino*butyrate
13.	Putrescine

Appendix C, Table 1. Results of boldface tests from the scheme of Baumann *et al.* (1984) for 118 study strains

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
1	-	-	-	+	-	-	-	-	+	+	+	-	-
2	-	-	-	+	-	-	-	-	+	+	+	-	-
3	-	-	-	+	-	-	-	-	+	+	+	-	-
4	-	-	-	+	-	-	-	-	+	+	+	-	-
5	-	-	-	+	-	-	-	-	+	+	+	-	-
6	-	-	-	+	-	-	-	-	+	+	+	-	-
7	-	-	-	+	-	-	-	-	+	+	+	-	-
8	-	-	-	+	-	-	-	-	+	+	+	-	-
9	-	-	-	+	-	-	-	-	+	+	-	-	-
10	-	-	-	w+	-	-	-	-	-	-	-	-	-
11	-	-	-	+	-	-	-	-	-	-	-	-	-
12	-	-	-	+	-	-	-	+	+	-	-	-	-
13	-	-	-	+	-	-	-	-	+	-	+	-	-
14	-	-	-	+	-	-	-	-	+	+	+	-	-
15	-	-	-	w+	-	-	-	-	-	-	-	-	-
16	-	-	-	+	-	-	-	-	-	-	-	-	-
17	-	-	-	+	+	-	-	-	-	-	-	-	-
18	-	-	-	+	+	-	-	-	-	-	-	-	-
19	-	-	-	+	+	-	-	-	-	-	-	-	-
20	-	-	-	+	+	-	-	+	-	-	-	-	-
21	-	-	+	+	+	-	-	-	-	-	-	-	
22	-	-	+	+	+	-	-	-	-	-	-	-	-
23	-	-	+	+	+	-	-	-	-	-	-	-	-
24	-	-	+	+	+	-	-	-	-	-	-	-	-
25	-	-	+	+	+	-	-	-	-	-	-	-	-
26	-	-	+	+	+	-	-	-	-	-	-	-	-
27	-	-	+	+	+	-	-	-	-	-	-	-	-
28	-	-	+	+	+	-	-	-	-	-	-	-	-
29	-	-	-	+	+	-	-	-	-	-	-	-	-

Appendix C, Table 1 continued:

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
30	-	-	-	+	+	-	-	-	-	-	-	-	-
31	-	-	-	+	+	-	-	-	-	-	-	-	-
32	-	-	+	+	+	-	-	-	-	-	-	-	-
33	-	-	+	+	+	-	-	-	-	-	-	-	-
34	-	-	+	+	-	-	-	-	-	-	-	-	-
35	-	-	+	+	-	-	-	-	-	-	-	-	-
36	-	-	+	+	+	-	-	-	-	-	-	-	-
37	-	-	-	+	+	-	-	-	-	-	-	-	-
38	-	-	-	+	+	-	-	-	-	-	-	-	-
39	-	-	-	+	+	-	-	-	-	-	-	-	-
40	-	-	-	+	+	-	-	-	-	-	-	-	-
41	-	-	-	+	+	-	-	-	+	+	-	-	+
42	-	-	-	+	+	-	-	+	+	+	-	-	+
43	-	-	-	+	+	-	-	+	+	+	-	-	+
44	-	-	-	+	+	-	-	-	+	-	-	-	+
45	-	-	-	+	+	-	-	-	+	-	-	-	-
46	-	-	-	+	+	-	-	-	+	W+	-	-	-
47	-	-	-	+	-	-	-	-	+	+	-	+	-
48	-	-	-	+	-	-	-	+	+	+	+	+	+
49	-	-	-	+	-	-	-	-	+	+	+	+	+
50	-	-	-	+	-	-	-	+	-	+	+	+	-
51	-	-	-	+	-	-	-	-	+	+	+	-	-
52	-	-	-	+	-	-	-	-	+	+	+	+	-
53	-	-	-	+	+	-	-	+	-	+	+	+	-
54	-	-	+	+	+	-	-	-	-	+	+	-	-
55	-	-	-	+	-	-	-	-	-	+	+	+	-
56	-	-	-	+	-	-	-	+	-	+	+	+	-
57	-	-	+	+	+	-	-	+	+	+	+	+	+
58	-	-	+	+	+	-	-	-	-	+	+	-	-
59	-	-	-	+	+	-	-	-	-	+	+	-	-
60	-	-	-	+	+	-	-	-	-	+	+	-	-

Appendix C, Table 1 continued:

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
61	-	-	-	+	+	-	-	+	-	+	-	-	-
62	-	-	+	+	+	-	-	-	-	+	-	-	-
63	-	-	+	+	+	-	-	-	-	+	-	-	-
64	-	-	+	+	+	-	-	-	+	+	+	-	-
65	-	-	+	+	+	-	-	-	w+	+	-	-	-
66	-	-	-	+	+	-	-	-	+	+	-	-	w+
67	-	-	-	+	+	-	-	-	+	+	+	-	w+
68	-	-	-	+	-	-	-	-	-	-	-	w+	-
69	-	-	-	+	+	-	-	-	+	+	-	-	w+
70	-	-	-	+	+	-	-	-	+	+	-	-	w+
71	-	-	-	+	+	-	-	-	+	+	-	-	w+
72	-	-	-	+	-	-	-	-	-	-	-	+	w+
73	-	-	-	+	-	-	-	+	-	-	w+	-	-
74	-	-	-	+	+	-	-	+	+	-	+	+	w+
75	-	-	-	+	+	-	-	+	+	-	+	+	w+
76	-	-	-	+	+	-	-	+	+	w+	-	-	w+
77	-	-	-	+	+	-	-	+	+	+	+	+	w+
78	-	-	-	+	+	-	-	+	-	-	+	-	-
79	-	-	-	+	+	-	-	-	-	-	-	-	-
80	-	-	-	+	+	-	-	-	+	-	-	-	-
2588	-	-	-	+	+	+	-	-	+	-	-	+	+
7744	-	-	-	+	+	-	-	-	-	+	-	-	-
9031	-	-	+	+	+	-	-	-	+	-	+	+	+
9067	-	-	-	+	+	-	+	+	-	-	-	-	-
9578	-	-	-	-	+	-	+	+	+	-	+	-	-
14048	-	-	-	+	+	-	-	+	+	-	+	+	+
14635	-	-	-	+	+	-	-	+	-	-	-	-	-
14715	-	-	+	+	+	+	+	-	+	-	+	+	+
15338	+	-	+	+	+	+	+	+	+	-	+	-	+
15381	-	-	-	+	+	-	-	-	-	-	+	-	-
15468	-	-	+	+	+	-	-	+	+	+	+	+	+

Appendix C, Table 1 continued:

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
17749	+	-		+	+	-	+	+	+	-	-	-	+
17802	-	-	-	+	+	-	-	+	+	-	+	-	+
19109	w+	-	+	+	+	-	-	+	+	+	+	-	+
19264	-	-	+	+	+	-	+	-	+	+	+	-	-
23211	-	-	+	+	+	-	+	+	+	-	+	+	+
25914	-	-	+	+	-	-	-	-	-	+	-	-	-
25916	-	-	+	+	+	-	-	+	-	-	-	+	+
25917	+	-	+	+	+	-	-	+	+	-	+	+	+
25919	-	-	-	+	+	-	-	-	-	+	+	+	+
25920	-	-	-	+	+	-	-	-	-	+	-	-	-
27013	-	-	+	+	-	-	+	-	+	-	+	+	+
27043	-	-	-	+	+	-	-	-	-	+	-	-	-
27562	-	-	-	+	+	-	-	+	-	+	+	-	-
29988	-	+	-	-	-	+	+	+	+	+	-	-	-
33125	-	-	+	+	-	-	-	-	-	+	-	-	-
33466	-	-	+	+	+	-	-	+	+	+	+	-	+
33509	-	-	-	+	-	-	-	-	+	-	-	-	-
33564	-	-	-	+	+	-	-	+	-	-	+	-	-
33653	-	-	-	+	+	-	-	+	-	-	+	-	-
33809	-	-	-	+	+	-	-	+	+	+	+	+	-
33934	-	-	+	+	+	-	-	-	+	+	+	-	+
35016	-	-	+	+	+	+	-	+	+	-	+	+	+
35048	-	-	+	+	+	-	-	-	+	+	+	+	+
35084	+	-	-	+	+	-	-	+	+	+	+	-	-
35912	-	-	-	+	+	-	-	+	+	+	+	+	-
43341	-	-	+	+	+	-	-	-	+	+	+	-	+
43979	-	-	+	+	+	+	+	-	-	-	-	+	+

Appendix C, Table 2. Growth temperature results for 118 study strains

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

Appendix C, Table 2 continued:

Strain	4°C	20°C	30°C	35°C
59	+	+	-	-
60	-	+	-	-
61	+	+	+	+
62	+	+	+	-
63	+	+	+	-
64	+	+	-	-
65	+	+	-	-
66	+	+	+	-
67	+	+	+	-
68	+	+	+	-
69	+	+	+	-
70	+	+	+	-
71	+	+	+	-
72	+	+	+	-
73	+	+	+	+
74	+	+	+	+
75	+	+	+	+
76	+	+	+	+
77	+	+	+	+
78	+	+	+	+
79	+	+	+	+
80	+	+	+	-
2588	-	+	+	+
7744	-	+	+	-
9031	-	+	+	+
9067	-	+	+	+
9578	+	+	+	+
14048	-	+	+	+
14635	-	+	+	+
14715	+	+	+	+

Strain	4°C	20°C	30°C	35°C
15338	-	+	+	+
15381	+	+	-	-
15468	+	+	+	+
17749	-	+	+	+
17802	-	+	+	+
19109	-	+	+	+
19264	+	+	+	+
23211	+	+	+	+
25914	-	+	+	-
25916	-	+	+	+
25917	-	+	+	+
25919	-	+	+	+
25920	-	+	+	+
27013	+	+	+	+
27043	-	+	+	+
27562	-	+	+	+
29988	-	+	+	+
33125	-	+	+	+
33466	+	+	+	+
33509	-	+	+	-
33564	-	+	+	+
33653	-	+	+	+
33809	-	+	+	+
33934	-	+	+	+
35016	-	+	+	+
35048	+	+	+	+
35084	-	+	+	+
35912	-	+	+	+
43341	-	+	+	+
43979	+	+	+	+

Appendix C, Table 3. Production of Chitinase results for 118 study strains

Strain	Result	Strain	Result	Strain	Result
1	-	30	+	59	-
2	-	31	+	60	-
3	-	32	-	61	-
4	-	33	-	62	+
5	-	34	-	63	+
6	-	35	+	64	+
7	-	36	+	65	+
8	-	37	-	66	+
9	-	38	+	67	-
10	-	39	+	68	-
11	-	40	-	69	+
12	-	41	-	70	-
13	-	42	-	71	-
14	-	43	-	72	-
15	-	44	-	73	-
16	-	45	+	74	-
17	-	46	-	75	-
18	-	47	-	76	-
19	-	48	-	77	-
20	-	49	-	78	-
21	+	50	-	79	-
22	+	51	-	80	-
23	-	52	-	14048	-
24	+	53	-	14635	-
25	-	54	+	14715	+
26	+	55	-	15338	+
27	+	56	-	15468	+
28	+	57	+	17749	+
29	-	58	+	17802	-

Appendix C, Table 3 continued:

Strain	Result	Strain	Result
19109	+	15381	+
19264	+		
23211	+		
2588	-		
25914	+		
25916	-		
25917	+		
25919	-		
25920	+		
27013	+		
27043	-		
27562	+		
29988	-		
33125	w+		
33466	-		
33509	-		
33564	+		
33653	+		
33809	-		
33934	w+		
35016	w+		
35048	-		
35084	+		
35912	+		
43341	-		
43979	-		
7744	-		
9031	-		
9067	-		
9578	-		

List for Appendix C, Table 4.

List of carbon compounds tested as sole source of carbon and energy, and positive and negative controls associated with these tests [Test no.'s lined up across top of Appendix C, Table 4]

Test no.	Control Medium
1	BASW [Negative control; test for growth because of trace nutrients]
2	YEPN [Positive control; test for viability]

Test no.	Carbon Compound	Test no.	Carbon Compound
3	Propionate	20	L-Ornithine
4	DL-Malate	21	L-Citrulline
5	L-Serine	22	D-Xylose
6	L-Alanine	23	L-Arabinose
7	β-Alanine	24	D-Mannose
8	D-Alanine	25	D-Galactose
9	L-Leucine	26	D-Trehalose
10	L-Tyrosine	27	α-Lactose
11	Malonate	28	D-Melibiose
12	L-Glutamate	29	D-Glucuronate
13	L-Valine	30	Salicin
14	Succinate	31	D-Galacturonate
15	Fumarate	32	Citrate
16	L-Tartrate	33	α-*Keto*glutarate
17	DL-Glycerate	34	Sodium Pyruvate
18	Glycine	35	Ethanol
19	DL-Aspartate	36	Propanol

List for Appendix C, Table 4 continued:

Test no.	Carbon Compound	Test no.	Carbon Compound
37	D-Mannitol	58	*iso*Butyrate
38	D-Sorbitol	59	Glycerol
39	*meso*-Inositol	60	*cis*-Aconitate
40	*p*-Hydroxybenzoate	61	L-Threonine
41	L-Histidine	62	D-Quinate
42	L-Proline	63	Benzoate
43	L-Rhamnose	64	Hydroxymethylglutarate
44	Sarcosine	65	Asparagine
45	Betaine	66	Adenine
46	Hippurate	67	Xanthine
47	*n*-Acetylglucosamine	68	Caprate
48	D-Ribose	69	Caprylate
49	D-Glucose	70	Caproate
50	D-Fructose	71	Ethanolamine
51	Maltose	72	Glutarate
52	Valerate	73	*meso*-Erythritol
53	Heptanoate	74	Phenylacetate
54	DL-Lactate	75	Pelargonate
55	DL-β-Hydroxybutyrate		
56	Acetate		
57	*iso*Valerate		

Appendix C, Table 4. Three-week carbon source utilization results for 118 study strains

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

Appendix C, Table 4 continued:

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

Appendix C, Table 4 continued:

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2588	-	+	+	+	+	+	w+	+	+	+	+	+	+	+	+	w+	+	+	+	+
7744	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
9031	-	+	+	+	w+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
9067	-	+	+	w+	w+	w+	-	-	w+	+	-	-	-	-	-	-	-		+	+
9578	-	+	+	w+	w+	+	-	+	+	+	-	+	+	+	+	-	-		+	+
14048	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
14635	-	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14715	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	w+
15338	-	+	+	+	+	+	+	+	w+	+	-	+	-	+	+	-	+	+	+	+
15381	-	+	-	+	+	-	-	+	-	-	-	+	-	+	+	-	-	-	+	-
15468	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	w+
17749	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
17802	-	+	+	w+	+	+	-	+	+	-	-	+	-	+	+	-	+	+	+	+
19109	-	+	+	+	+	+	+	+	w+	+	-	+	-	+	+	-	+	+	+	+
19264	-	+	-	+	+	+	-	+	-	-	-	+	-	+	+	-	+		w+	
23211	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	-
25914	-	+	+	+	+	+	-	+	-	+	-	w+	-	+	+	-	-	+	w+	+
25916	-	+	+	w+	-	+	-	+	+	+	-	+	+	+	+	-	-	-	+	+
25917	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
25919	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25920	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-		w+	+	+
27013	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
27043	-	+	+	w+	+	+	+	+	-	w+	-	+	-	+	+	-	w+	+	+	+
27562	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	+		+	+
29988	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	
33125	-	+	+	-	-	+	w+	+	+	+	-	+	+	+	+	-	+		+	+
33466	-	+	+	+	+	+	w+	+	+	+	-	+	+	+	+	-	+	-	+	+
33509	-	w+	+	+	+	+	w+	+	+	+	-	+	+	+	+	-	+	+	+	+
33564	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
33653	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
33809	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
33934	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
35016	-	+	+	+	+	+	w+	+	+	+	-	+	+	+	+	-	+	+	+	+
35048	-	+	+	+	+	+	w+	+	+	+	+	+	+	+	+	+	+	+	+	+
35084	-	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	-
35912	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-
43341	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+	+
43979	-	+	+	+	w+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	-

Appendix C, Table 4 continued:

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

Appendix C, Table 4 continued:

Strain	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
2588	w+	-	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-
7744	-	w+	-	-	-	-	-	w+	-	-	-	-	-	-	-	-	-	-	w+	w+
9031	w+	-	+	-	-	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-
9067	w+	-	-	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+	w+	-
9578	w+	-	-	+	-	+	-	-	-	w+	-	+	-	+	-	-	+	-	+	-
14048	+	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	-	-	+
14635	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14715	-	-	-	+	-	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-
15338	+	-	-	+	-	+	-	-	-	w+	-	+	-	+	-	+	+	+	-	-
15381	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
15468	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-
17749	-	-	+	-	+	+	-	-	-	+	-	+	-	+	-	w+	+	-	-	-
17802	-	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	+	-	-
19109	+	-	-	+	-	+	-	-	-	w+	-	+	-	+	+	+	+	+	-	-
19264	w+	-	+	+	-	+	w+	-	-	-	-	-	-	+	-	-	+	+	-	-
23211	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	-	+	+	-	-
25914	w+	-	-	w+	-	+	w+	-	-	-	-	-	-	+	-	-	+	-	-	-
25916	w+		-	w+	-	+	w+	-	-	-	-	+	-	+	-	-	-	-	-	-
25917	+	-	+	+	-	+	w+	+	-	-	-	+	-	+	+	+	+	+	-	-
25919	+	+	+	+	-	+	w+	+	+	-	+	+	-	+	+	+	+	+	-	-
25920	w+	-	-	w+	-	+	w+	-	-	-	-	-	-	+	-	-	+	-	-	-
27013	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
27043	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	-	+	+	+	-
27562	+	-	-	w+	+	+	w+	-	-	-	-	+	-	+	-	-	-	-	-	-
29988	-	+	+	+	-	+	w+	-	+	+	-	w+	-	+	-	-	+	-	-	-
33125	+	w+	+	-	-	-	w+	-	-	-	-	+	-	+	+	+	-	-	-	-
33466	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-
33509	+	w+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
33564	+	w+	+	+	-	+	-	-	-	w+	-	+	-	+	+	+	+	+	-	-
33653	+	w+	+	+	+	+	-	-	-	w+	-	+	-	+	+	+	+	-	-	-
33809	+	w+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
33934	+	w+	w+	+	-	+	-	-	w+	w+	-	+	-	+	+	+	+	-	-	-
35016	+	w+	+	+	+	+	-	-	w+	w+	+	+	-	+	+	+	+	-	-	+
35048	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	w+	-
35084	w+	+	+	w+	+	+	-	-	+	+	-	+	-	+	-	+	+	-	+	-
35912	w+	+	+	+	+	+	-	-	+	+	-	+	-	+	-	-	+	-	+	-
43341	w+	+	+	w+	+	+	+	+	w+	+	-	+	-	+	-	-	+	+	+	-
43979	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-

Appendix C, Table 4 continued:

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

Appendix C, Table 4 continued:

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

Appendix C, Table 4 continued:

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

Appendix C, Table 4 continued·

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

Appendix C, Table 4 continued:

Strain	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
2588	+	+	w+	w+	+	w+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
7744	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	w+	w+	-
9031	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
9067	w+	-	-	-	w+	-	+	+	+	+	+	+	+	+	-	-	-	-	+	-
9570	+	+	-	-	w+	-	+	+	+	+	+	+	+	+	+	+	w+	+	+	-
14048	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	w+	+	+	-
14635	-	-	-	-	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
14715	+	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-
15338	+	+	-	-	w+	-	+	+	+	+	+	-	+	+	-	+	-	-	+	+
15381	-	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	-
15468	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-
17749	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	
17802	+	+	-	-	w+	-	+	+	+	+	+	-	+	+	-	+	-		+	
19109	+	+	-	-	w+	-	+	+	+	+	+	-	+	+	-	+	-		+	+
19264	-	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-		+	
23211	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	
25914	-	+	-	-	-	-	+	+	w+	+	+	-	w+	+	-	+	-	-	+	-
25916	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
25917	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25919	+	+	-	w+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
25920	-	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	
27013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	
27043	+	+	-	-	-	-	+	+	-	-	+	-	+	+	+	w+	-	-	+	-
27562	+	+	-	-	-	-	+	+	-	+	+	-	+	+	-	w+	-	-	+	-
29988	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	w+	-	-	+	-
33125	+	+	-	-	-	-	+	-	+	-	w+	-	-	-	-	-	-	-	-	-
33466	+	+	w+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
33509	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-		-
33564	-	+	-	-	-	-	+	-	+	+	+	-	+	+	-	+	-	-	+	-
33653	+	+	-	-	-	-	+	+	+	+	+	-	w+	+	-	+	-	-	+	-
33809	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+
33934	-	+	w+	-	-	w+	+	-	+	+	+	-	+	+	+	+	-	-	+	
35016	+	+	w+	-	-	w+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
35048	+	+	w+	-	-	+	+	+	-	+	+	-	+	+	-	-	-	-	+	
35084	+	+	-	-	-	-	+	+	-	-	+	-	+	+	-	+	-	-	+	-
35912	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	+	-	-	+	-
43341	+	+	-	-	-	-	+	+	-	-	+	-	+	+	w+	+	+	-	+	-
43979	+	+	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	-

Appendix C, Table 4 continued:

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

Appendix C, Table 4 continued:

Strain	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
2588	-	-	-	-	+	-	w+	+	+	+	-	+	-	-	+
7744	-	-	-	-	+	-	-	-	w+	+	-	-	-	-	w+
9031	-	-	-	-	+	-	-	+	+	+	-	+	-	+	+
9067	-	-	-		-	-	-	+	+	+	-	-	-	-	-
9578	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
14048	+	+	+	-	+	-	-	+	+	+	-	+	-	w+	+
14635	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
14715	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
15338	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+
15381	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
15468	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+
17749	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
17802	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
19109	+	-	-	-	+	+	w+	+	+	+	-	-	-	-	+
19264	-	-	-	-	+	-	-	+	w+	-	-	-	-	-	-
23211	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
25914	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
25916	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
25917	+	-	-	-	+	+	w+	+	+	+	-	-	-	-	+
25919	w+	-	-	w+	+	-	w+	+	+	+	-	+	-	w+	+
25920	+	-	-	-	+	-	-	+	+	+	-	-	-	-	+
27013	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
27043	+	-	-	-	+	-	-	-	w+	+	-	-	+	-	-
27562	+	-	-	-	+	-	-	+	w+	+	-	-	-	-	-
29988	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
33125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33466	+	-	-	-	+	-	-	+	+	+	-	+	-	-	+
33509	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33564	w+	+	-	-	w+	-	-	+	+	w+	-	-	-	-	-
33653	w+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
33809	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+
33934	+	-	-	-	+	+	-	+	+	-	-	-	-	-	-
35016	+	+	w+	-	+	-	-	+	+	+	-	+	-	-	+
35048	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
35084	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
35912	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
43341	+	w+	-	-	+	-	-	+	+	+	-	-	-	-	-
43979	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+

APPENDIX D

List, Appendix D: List of carbon compounds tested as soie source of carbon and energy, and positive and negative controls associated with these tests [Test no.'s lined up across top of Appendix D]

Test no.	Control Medium
1	BASW [Negative control; test for growth because of trace nutrients]
2	YEPN [Positive control; test for viability]

Test no.	Carbon Compound	Test no.	Carbon Compound
3	Propionate	20	L-Ornithine
4	DL-Malate	21	L-Citrulline
5	L-Serine	22	D-Xylose
6	L-Alanine	23	L-Arabinose
7	β-Alanine	24	D-Mannose
8	D-Alanine	25	D-Galactose
9	L-Leucine	26	D-Trehalose
10	L-Tyrosine	27	α-Lactose
11	Malonate	28	D-Melibiose
12	L-Glutamate	29	D-Glucuronate
13	L-Valine	30	Salicin
14	Succinate	31	D-Galacturonate
15	Fumarate	32	Citrate
16	L-Tartrate	33	α-*Keto*glutarate
17	DL-Glycerate	34	Sodium Pyruvate
18	Glycine	35	Ethanol
19	DL-Aspartate	36	Propanol

List, Appendix D continued:

Test no.	Carbon Compound	Test no.	Carbon Compound
37	D-Mannitol	58	*iso*Butyrate
38	D-Sorbitol	59	Glycerol
39	*meso*-Inositol	60	*cis*-Aconitate
40	*p*-Hydroxybenzoate	61	L-Threonine
41	L-Histidine	62	D-Quinate
42	L-Proline	63	Benzoate
43	L-Rhamnose	64	Hydroxymethylglutarate
44	Sarcosine	65	Asparagine
45	Betaine	66	Adenine
46	Hippurate	67	Xanthine
47	*n*-Acetylglucosamine	68	Caprate
48	D-Ribose	69	Caprylate
49	D-Glucose	70	Caproate
50	D-Fructose	71	Ethanolamine
51	Maltose	72	Glutarate
52	Valerate	73	*meso*-Erythritol
53	Heptanoate	74	Phenylacetate
54	DL-Lactate	75	Pelargonate
55	DL-β-Hydroxybutyrate		
56	Acetate		
57	*iso*Valerate		

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
14	1	-	+	+	-	+	+	-	-	-	+	-	+	-	+	+	-	-	-	+	-
14	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-		-	+	w+
14	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	+	w+
15	1	-	+	+	w+	+	+	-	+	+	+	-	+	-	+	+	w+	w+	-	+	-
1	2	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	w+	w+	+	+	w+
15	3	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	w+	+	+	+	w+
16	1	-	+	+	+	+	+	-	+	+	+	+	+	w+	+	+	w+	w+	+	+	
16	2	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	w+	w+	+	+	w+
16	3	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	w+	+	+	+	w+
17	1	-	+	+	-	+	w+	-	+	-	-	-	+	-	+	+	-		w+	+	-
17	2	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	-	+	+	
17	3	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	-	+	+	
18	1	-	+	w+	-	+	+	-	+	-	-	-	+	-	+	+	-	-	+	+	
18	2	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	w+	+	+	
18	3	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+		+	+	+	-
19	1	-	+	w+	-	+	+	-	+	-	-	-	+	-	+	+		-	+	+	
19	2	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	w+	+	+	
19	3	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	+	-
20	1	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+		-	+	+	
20	2	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	w+	+	+	w+
20	3	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	+	w+
21	1	-	+	-	-	+	+	-	+	-	w+	-	+	-	+	+	-	-	+	-	
21	2	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+	-	w+	+	+	w+
21	3	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	-	w+	+	+	w+
22	1	-	+	w+	-	+	-	-	+	-	-	-	+	-	+	+	-			+	
22	2	-	+	w+	-	+	+	-	+	-	-	-	+	-	+	+	-	w+	-	+	w+
22	3	-	+	w+	-	+	+	-	+	w+	-	-	+	-	+	+	-	w+		+	w+
23	1	-	+	w+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	-
23	2	-	+	w+	-	+	+	-	+	w+	-	-	+	-	+	+	-	+	+	+	
23	3	-	+	w+	-	+	+	-	+	w+	-	-	+	-	+	+	-	+	+	+	
24	1	-	+	w+	-	+	-	-	+	-	-	-	+	-	+	+	-	-	+	+	w+
24	2	-	+	w+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	+
24	3	-	+	w+	-	+	+	-	+	w+	-	-	+	-	+	+	-	+	+	+	+
25	1	-	+	w+	-	+	-	-	-	-	+	-	+	-	+	+	-	-		+	
25	2	-	+	w+	-	+	-	-	-	-	+	-	+	-	+	+	-	+		+	
25	3	-	+	w+	-	+	w+	-	-	w+	+	-	+	-	+	+	-	+	-	+	
26	1	-	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-			+	-
26	2	-	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	
26	3	-	+	+	-	+	-	-	-	w+	-	-	+	-	+	+	-	-		+	
27	1	-	+	w+	-	+	-	-	+	-	-	-	+	-	+	+	-	-	+	+	
27	2	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	w+	+	+	
27	3	-	+	+	-	+	+	-	+	w+	-	-	+		+	+	-	w+	+	+	

Appendix D. One, two, and three-week carbon source utilization results for 118 study strains

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	-	+	+	+	+	+	-	+	w+	+	-	+	-	+	+	-	-	+	w+	w+
1	2	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	+	+	w+
1	3	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	+	+	+
2	1	-	+	+	-	w+	+	-	-	w+	+	-	+	-	+	+	-	-	-	w+	w+
2	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	+	w+
2	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	+	w+
3	1	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	+
3	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	w+	+	+	+
3	3	-	+	+	-	+	+	-	-	+	+	-	+	w+	+	+	w+	w+	+	+	+
4	1	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	w+	-	w+	+
4	2	-	+	+	-	+	+	w+	-	+	+	-	+	-	+	+	-	+	+	+	+
4	3	-	+	+	-	+	+	w+	-	+	+	-	+	w+	+	+	-	+	+	+	+
5	1	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	+
5	2	-	+	+	-	+	+	w+	-	+	+	-	+	-	+	+	-	-	w+	+	+
5	3	-	+	+	-	+	+	w+	-	+	+	-	+	-	+	+	-	-	+	+	+
6	1	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	w+
6	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	w+	+	w+
6	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	+	+	w+
7	1	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	+
7	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	w+	+	+
7	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	+	+	+
8	1	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	w+
8	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	w+	+	w+
8	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	+	+	w+
9	1	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	w+
9	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	w+	+	w+
9	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	+	+	w+
10		-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	+
10	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	w+	+	+
10	3		+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	+	+	+
11	1	-	+	+	-	w+	+	-	-	+	+	-	+	-	+	+	-	-	-	w+	w+
11	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	w+	+	w+
11	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	+	+	w+
12	1	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	-	+	-
12	2	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	-	w+	-	+	w+
12	3	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	-	+	-	+	w+
13	1	-	+	+	-	+	+	-	-	-	+	-	+	-	+	+	-	-	-	+	-
13	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	+	w+
13	3	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	+	w+

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
28	1	-	+	w+	-	+	w+	-	+	-	-	-	+	-	+	+	-		+	+	-
28	2	-	+	+	-	+	+	-	+	-		-	+	-	+	+	-	w+	+	+	w+
28	3	-	+	+	-	+	+	-	+	w+	-	-	+	-	+	+	-	+	+	+	+
29	1	-	+	-		-	-	-	-	-	-	-	-	-	+	-	-		-		
29	2	-	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-				
29	3	-	+	+	-	-	-	-	-	w+	-		+	-	+		-	w+	-	-	
30	1	-	+	w+	-	+	-	-	+	-	-	-	+	-	+	+		w+	+	+	
30	2	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+	-	+	+	+	
30	3	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+		+	+	+	
31	1	-	+	w+	-	+	-	-	+	-	-	-	+	-	+	+	-	-		+	
31	2	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	
31	3	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	w+
32	1	-	+	w+	-	-	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	
32	2	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+	-	w+	+	+	w+
32	3	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+	-	+	+	+	+
33	1	-	+	w+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	
33	2	-	+	+	-	+	+	-	+	w+	-	-	+	-	+	+	-	w+	+	+	
33	3	-	+	+	-	+	+	-	+	w+	-	-	+	-	+	+	-	+	+	+	
34	1	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-			-
34	2	-	+	+	-	-	-	-	-	-	-	-	+	-	+	+		w+		-	
34	3	-	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	+			
35	1	-	+	-		-	-	-	-	-	-	-	w+	-	+	+	-	-	-	+	
35	2	-	+	+	-	-	+	-	-	-	-	-	+		+	+	-	w+	-	+	
35	3	-	+	+	-	-	+	-	-	-	-	-	+	-	+	+		+		+	
36	1	-	+	w+	-	w+	w+	-	+	-	-	-	+	-	+	+	-		+	+	
36	2	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+		w+	+	+	
36	3	-	+	+	-	+	+	-	+	w+	-	-	+	-	+	+	-	+	+	+	
37	1	-	+	w+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	
37	2	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	+	+	-
37	3	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+	-	+	+	+	+
38	1	-	+	w+	-	-	-	-	-	-	-	-	-	-	+	+	-				-
38	2	-	+	+	-	-	-	-	-	-	-	-	w+	-	+	+	-	w+		-	
38	3	-	+	+	-	w+	+	-	-	-	-	-	+	-	+	+		w+		-	w+
39	1	-	+	w+	-	-	-	-	-	-	-	-	-	-	+	+	-				
39	2	-	+	+	-	w+	-	-	-	-	-	-	w+	-	+	+		+	-	-	
39	3	-	+	+	-	+	w+	-	-	-	-	-	+	-	+	+	-	+	-		w+
40	1	-	+	w+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-		
40	2	-	+	+	-	+	-	-	-	-	-	-	w+	-	+	+	-	+	-		
40	3	-	+	+	-	+	+	-	-	-	-	-	+	-	+	+	-	+		-	w+
41	1	-	+	w+	-	-	-	-	-	-	-	-	w+	-	+	+	-	-			-
41	2	-	+	+	-	+	-	-	-	w+	-	-	+	-	+	+	-	+			-
41	3	-	-	+	-	+	+	-	-	w+	-	-	+	-	+	+	-	+			w+

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
42	1	-	+	w+	-	-	-	-	-	-	-	-	w+	-	+	+	-	w+	-	-	-
42	2	-	+	+	-	+	-	-	-	w+	-	-	+	-	+	+	-	+	-	-	-
42	3	-	+	+	-	+	+	-	-	w+	-	-	+	-	+	+	-	+	-	-	w+
43	1	-	+	w+	-	-	-	-	-	-	-	-	w+	-	+	+	-	w+	-	-	-
43	2	-	+	+	-	+	-	-	-	w+	-	-	+	-	+	+	-	+	-	-	-
43	3	-	+	+	-	+	+	-	-	w+	-	-	+	-	+	+	-	+	-	-	w+
44	1	-	+	w+	-	-	-	-	-	-	-	-	w+	-	+	+	-	w+	-	-	-
44	2	-	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-
44	3	-	+	+	-	+	+	-	-	-	-	-	+	-	+	+	-	+	-	-	w+
45	1	-	+	w+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
45	2	-	+	+	-	w+	-	-	-	-	-	-	+	-	+	+	-	w+	-	-	-
45	3	-	+	+	-	+	w+	-	-	-	-	-	+	-	+	+	-	w+	-	-	w+
46	1	-	+	w+	-	-	-	-	-	-	-	-	w+	-	+	+	-	w+	-	-	-
46	2	-	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-
46	3	-	+	+	-	w+	w+	-	-	-	-	-	+	-	+	+	-	+	-	-	w+
47	1	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+
47	2	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	w+	+	+
47	3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
48	1	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+
48	2	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	w+	+	+
48	3	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
49	1	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	-	+	+
49	2	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	w+	-	+	+
49	3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	w+	w+	+	+
50	1	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+
50	2	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
50	3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
51	1	-	+	+	-	w+	+	-	w+	-	+	-	+	-	+	+	-	-	-	w+	-
51	2	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+	-	-	-	w+	-
51	3	-	+	+	-	+	+	-	+	w+	+	-	+	-	+	+	-	-	w+	w+	-
52	1	-	+	+	-	w+	+	-	-	w+	+	-	+	-	+	+	-	-	w+	w+	-
52	2	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	-	w+	+	+	-
52	3	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	-	w+	+	+	-
53	1	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+
53	2	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
53	3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
54	1	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	-	+	+	-
54	2	-	+	+	-	+	+	-	+	w+	-	-	+	-	+	+	-	w+	+	+	-
54	3	-	+	+	-	+	+	-	+	w+	-	-	+	-	+	+	-	w+	+	+	-
55	1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	2	-	+	w+	-	w+	w+	-	w+	-	+	-	w+	-	+	+	-	-	-	w+	-
55	3	-	+	w+	-	w+	w+	-	+	w+	+	-	+	-	+	+	-	w+	-	+	-

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
56	1	-	+	w+	+	+	+	+	+	w+	+	+	+	-	+	+		+		+	+
56	2	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+		+	+
56	3	-	+	+	+	+	+	+	+	+	+	+	+	w+	+	+	-	+	+	+	+
57	1	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
57	2	-	+	+	+	+	+	+	+	+	+	+	+	w+	+	+	-	+	+	+	+
57	3	-	+	+	+	+	+	+	+	+	+	+	+	w+	+	+	-	+	+	+	+
58	1	-	+	+	-	+	-	-	+	-	-	-	+	-	+	+	-	w+	+	+	w+
58	2	-	+	+	-	+	-	-	+	-	w+	-	+	-	+	+	-	w+	+	+	+
58	3	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	+	+	+
59	1	-	+	+	-	+	-	-	+	-	-	-	+	-	+	+	-	-	+	+	
59	2	-	+	+	-	+	-	-	+	-	w+	-	+	-	+	+	-	w+	+	+	-
59	3	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	-	w+	+	+	+
60	1	-	+	+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	-
60	2	-	+	+	-	+	+	-	+	-	w+	-	+	-	+	+	-	w+	+	+	-
60	3	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	+	+	-
61	1	-	+	+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	
61	2	-	+	+	-	+	+	-	+	-	w+	-	+	-	+	+	-	w+	+	+	
61	3	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	+	+	-
62	1	-	+	+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	-
62	2	-	+	+	-	+	+	-	+	-	w+	-	+	-	+	+		w+	+	+	-
62	3	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	+	+	w+
63	1	-	+	+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	
63	2	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	w+	+	+	-
63	3	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	+	+	w+
64	1	-	+	+	-	+	w+	-	+	-	-	-	+	-	+	+	-		+	+	
64	2	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+		w+	+	+	-
64	3	-	+	+	-	+	+	-	+	-	+	-	+	-	+	+	-	w+	+	+	-
65	1	-	+	+	-	+	w+	-	+	-	-	-	+	-	+	+	-	-	+	+	
65	2	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	w+	+	+	-
65	3	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	w+	+	+	w+
66	1	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-		+	
66	2	-	+	+	-	+	+	-	-	+	-	-	+	-	+	+	-	+	-	+	
66	3	-	+	+	-	+	+	-	w+	+	+	-	+	-	+	+	-	+	w+	+	w+
67	1	-	+	+	-	w+	-	-	-	+	-	-	+	-	+	+	-	+	-	+	-
67	2	-	+	+	-	+	w+	-	-	+	-	-	+	-	+	+	-	+		+	
67	3	-	+	+	-	+	w+	-	w+	+	+	-	+	-	+	+	-	+	-	+	-
68	1	-	+	+	-	-	-	-	+	-	w+	-	+	-	+	+	-	-		+	
68	2	-	+	+	-	-	w+	-	+	w+	+	-	+	-	+	+	-	-	-	+	
68	3	-	+	+	-	w+	+	-	+	+	+	-	+	-	+	+	-	-	-	+	-
69	1	-	+	w+	-	w+	-	-	-	-	-	-	+	-	w+	+	-	+	-	-	
69	2	-	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-
69	3	-	+	+	-	+	+	-	-	+	-	-	+	-	+	+	-	+	-	-	-

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
70	1	-	+	+	-	-	-	-	-	w+	-	-	+	-	w+	+	-	+	-	-	-
70	2	-	+	+	-	+	-	-	-	+	-	-	+	-	+	+	-	+	-	-	-
70	3	-	+	+	-	+	+	-	-	+	w+	-	+	-	+	+	-	+	-	-	-
71	1	-	+	w+	-	-	-	-	-	-	-	-	+	-	w+	+	-	+	-	-	-
71	2	-	+	+	-	+	-	-	-	+	-	-	+	-	+	+	-	+	-	-	-
71	3	-	+	+	-	+	+	-	-	+	w+	-	+	-	+	+	-	+	-	-	-
72	1	-	+	-	-	-	-	-	-	-	+	-	+	-	w+	+	-	-	-	-	-
72	2	-	+	+	-	-	-	-	+	+	+	-	+	-	+	+	-	-	-	-	-
72	3	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	-	-	-	-	-
73	1	-	+	-	-	-		-	-	-	+	-	w+	-	w+	+	-	-	-	-	-
73	2	-	+	+	-	-	-	-	+	+	+	-	+	-	+	+	+	+	-	-	+
73	3	-	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	+
74	1	-	+	+	-	-	+	-	+	w+	+	+	+	-	w+	+	-	w+	+	-	-
74	2	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	w+
74	3	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	w+
75	1	-	+	+	-	-	+	-	+	w+	+	+	+	-	w+	+	-	-	+	-	-
75	2	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	-	+	+	-	-
75	3	-	+	+	-	-	+	w+	+	+	+	+	+	+	+	+	-	+	+	-	-
76	1	-	+	+	-	w+	-	-	-	-	-	-	+	-	w+	+	+	w+	-	-	-
76	2	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	+	+	w+	-	-
76	3	-	+	+	-	+	+	+	-	+	+	-	+	-	+	+	+	+	w+	-	-
77	1	-	+	+	-	-	+	-	+	w+	+	+	+	-	w+	+	-	-	+	-	-
77	2	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	-	+	+	-	w+
77	3	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	w+
78	1	-	+	-	-	-	w+	-	+	-	+	-	+	-	w+	+	-	-	-	-	-
78	2	-	+	+	-	-	+	-	+	-	+	-	+	-	+	+	-	+	+	+	-
78	3	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	w+	+	+	+	-
79	1	-	+	w+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
79	2	-	+	+	-	+	+	-	-	-	-	-	+	-	+	+	-	+	-	+	-
79	3	-	+	+	-	+	+	-	-	-	-	-	+	-	+	+	-	+	-	+	-
80	1	-	+	w+	-	-	-	-	-	-	-	-	+	-	w+	+	-	-	-	-	-
80	2	-	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	+	-	+	-
80	3	-	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	+	-	+	-
14048	1	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
14048	2	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
14048	3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
14635	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14635	2	-	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14635	3	-	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14715	1	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	-
14715	2	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	-
14715	3	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	w+

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
15338	1	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+	+
15338	2	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+	+
15338	3	-	+	+	+	+	+	+	+	W+	+	-	+	-	+	+	-	+	+	+	+
15468	1	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	-
15468	2	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	-
15468	3	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	W+
17749	1	-	+	+	+	+	+	-	+	-	-	+	+	+	+	+	-	-	-	-	
17749	2	-	+	+	+	+	+	-	+	+	W+	+	+	+	+	+	-	-	-	W+	+
17749	3	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+		+	+	+	+
17802	1	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	+	-	+	-
17802	2	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	+	W+
17802	3	-	+	+	W+	+	+	-	+	+	-	-	+	-	+	+	-	+	+	+	+
19109	1	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	-	+	+	+
19109	2	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	-	+	+	+
19109	3	-	+	+	+	+	+	+	+	W+	+	-	+	-	+	+	-	+	+	+	+
19264	1	-	+	-	-	+	-	-	-	-	-	-	W+	-	+	+	-	+	-	-	-
19264	2	-	+	-	W+	+	+	-	+	-	-	-	W+	-	+	+	-	+	-	-	-
19264	3	-	+	-	+	+	+	-	+	-	-	-	+	-	+	+	-	+	-	W+	-
23211	1	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+	-	+	-	+	-
23211	2	-	+	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	
23211	3	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+		+	-
2588	1	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	W+
2588	2	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
2588	3	-	+	+	+	+	+	W+	+	+	+	+	+	+	+	+	W+	+	+	+	+
25914	1	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+	-	-	+	-	-
25914	2	-	+	+	W+	+	+	-	+	-	+	-	-	-	+	+	-	-	+	-	+
25914	3	-	+	+	+	+	+	-	+	-	+	-	W+	-	+	+	-	-	+	W+	+
25916	1	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-		-	+	-
25916	2	-	+	+	-	-	+	-	+	W+	+	-	+	+	+	+	-	-	-	+	+
25916	3	-	+	+	W+	-	+	-	+	+	+	-	+	+	+	+	-	-	-	+	+
25917	1	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	W+	+
25917	2	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	W+	+	+	+	+
25917	3	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
25919	1	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
25919	2	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25919	3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25920	1	-	+	-	-	-	+	-	+	-	+	-	+	-	+	+	-	-		W+	
25920	2	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	-	W+	W+
25920	3	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	W+	+	+
27013	1	-	W+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27013	2	-	W+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27013	3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
27043	1	-	+	+	-	+	+	+	+	-	-	-	+	-	+	+	-	-	-	w+	-
27043	2	-	+	+	-	+	+	+	+	-	-	-	+	-	+	+	-	-	+	w+	w+
27043	3	-	+	+	w+	+	+	+	+	-	w+	-	+	-	+	+	-	w+	+	+	+
27562	1	-	+	+	+	-	+	-	+	-	-	-	w+	-	+	+	-	+	-	+	-
27562	2	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	+	-	+	w+
27562	3	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	+	-	+	+
29988	1	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	w+	-
29988	2	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-
29988	3	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-
33125	1	-	+	-	-	-	-	-	+	-	-	-	+	-	+	+	-	+	-	+	+
33125	2	-	+	+	-	-	w+	-	+	+	+	-	+	+	+	+	-	+	-	+	+
33125	3	-	+	+	-	-	+	w+	+	+	+	-	+	+	+	+	-	+	-	+	+
33466	1	-	+	+	-	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	+
33466	2	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	+
33466	3	-	+	+	+	+	+	w+	+	+	+	-	+	+	+	+	-	+	-	+	+
33509	1	-	w+	+	-	+	+	-	+	+	+	-	+	+	+	+	-	+	w+	+	+
33509	2	-	w+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+
33509	3	-	w+	+	+	+	+	w+	+	+	+	-	+	+	+	+	-	+	+	+	+
33564	1	-	+	+	-	+	+	-	+	-	-	-	+	+	+	+	-	+	-	+	+
33564	2	-	+	+	+	+	+	-	+	-	w+	-	+	+	+	+	-	+	+	+	+
33564	3	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
33653	1	-	+	+	-	+	+	-	+	-	-	-	+	+	+	+	-	+	-	+	+
33653	2	-	+	+	+	+	+	-	+	-	w+	-	+	+	+	+	-	+	+	+	+
33653	3	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
33809	1	-	+	+	w+	+	+	-	+	-	-	-	+	+	+	+	-	+	-	+	+
33809	2	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	-	+	+	+	+
33809	3	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
33934	1	-	+	+	-	+	+	-	+	-	-	-	+	+	+	+	-	+	-	+	+
33934	2	-	+	+	w+	+	+	-	+	-	w+	-	+	+	+	+	-	+	+	+	+
33934	3	-	+	+	+	+	+	w+	+	-	+	-	+	+	+	+	-	+	+	+	+
35016	1	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	+	+	+	+
35016	2	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+
35016	3	-	+	+	+	+	+	w+	+	+	+	-	+	+	+	+	-	+	+	+	+
35048	1	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+
35048	2	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
35048	3	-	+	+	+	+	+	w+	+	+	+	+	+	+	+	+	+	+	+	+	+
35084	1	-	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	-	-	-
35084	2	-	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	w+	+	+	-
35084	3	-	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	-
35912	1	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	w+	-
35912	2	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-
35912	3	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-

Appendix D continued:

Strain	Wk	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
43341	1	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	+	+	W+	
43341	2	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+		+	+	+	W+
43341	3	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+	+
43979	1	-	+	+	W+	-	+	-	+	+	+	-	+	+	+	+	-	+	-	+	
43979	2	-	+	+	W+	-	+	-	+	+	+	-	+	+	+	+	-	+		+	
43979	3	-	+	+	+	W+	+	-	+	+	+	-	+	+	+	+		+		+	-
7744	1	-	+	-	-	-	-	-	-	-	-	-	-	+		-	-				
7744	2	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-		-
7744	3	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-		-	
9031	1	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+		-	+	+	+
9031	2	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
9031	3	-	+	+	+	W+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
9067	1	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-			+
9067	2	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	W+	+
9067	3	-	+	+	W+	W+	W+	-	-	W+	+	-	-	-	-	-	-	-	-	+	+
9578	1	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	-	-	+	
9578	2	-	+	+	-	-	+	-	+	+	+	-	+	+	+	+	-	-		+	W+
9578	3	-	+	+	W+	W+	+	-	+	+	+	-	+	+	+	+	-	-		+	+

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	1	+	+	-	-	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
1	2	+	+	+	+	+	w+	+	+	+	-	-	+	-	+	-	-	+	-	-	-
1	3	+	+	+	+	+	+	+	+	+	w+	-	+	-	+	-	w+	+	w+	w+	-
2	1	+	+	-	-	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
2	2	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
2	3	+	+	-	+	+	w+	+	+	-	-	-	+	-	+	-	-	+	w+	-	-
3	1	+	+	-	-	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
3	2	+	+	-	+	+	w+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
3	3	+	+	-	+	+	w+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
4	1	+	+	-	-	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
4	2	+	+	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
4	3	+	+	-	+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	w+	w+	w+
5	1	+	+	-	w+	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
5	2	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
5	3	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
6	1	+	+	-	-	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
6	2	+	+	-	+	+	w+	+	+	-	-	-	+	-	+	-	w+	+	-	-	-
6	3	+	+	-	+	+	+	+	+	-	-	-	+	-	+	-	w+	+	-	-	-
7	1	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-
7	2	+	+	-	+	+	+	+	+	-	-	-	-	-	+	w+	w+	+	-	-	-
7	3	+	+	-	+	+	+	+	+	-	-	-	-	-	+	w+	w+	+	w+	-	-
8	1	+	+	-	-	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
8	2	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
8	3	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-
9	1	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	+
9	2	+	-	-	+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	+
9	3	+	-	-	+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	+
10	1	+	+	-	-	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	+
10	2	+	+	-	+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	+
10	3	+	+	-	+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	+
11	1	+	+	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	+
11	2	+	+	-	+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	+
11	3	+	+	-	+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	+
12	1	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	+
12	2	+	-	-	+	+	+	+	+	-	w+	-	+	-	+	+	+	+	w+	w+	+
12	3	+	-	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	w+	+	+
13	1	+	+	w+	w+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	+
13	2	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	w+	+
13	3	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+
14	1	+	+	-	w+	+	+	+	+	-	-	-	+	-	+	w+	w+	+	-	-	-
14	2	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	w+	w+	+
14	3	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
15	1	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-	-	+		-	+
15	2	+	w+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	w+	+	+
15	3	+	w+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+
16	1	+	w+	+	+	+	+	+	+	-	-	w+	+	-	+	w+	+	+	+	w+	+
16	2	+	+	+	+	+	+	+	+	w+	+	w+	+	-	+	+	+	+	+	+	+
16	3	+	+	+	+	+	+	+	+	w+	+	w+	+	-	+	+	+	+	+	+	+
17	1	+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	-		-
17	2	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	w+	+	w+
17	3	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+
18	1	-	-	-	+	+	+	-	-	w+	-	-	+	-	+	-	-	+			-
18	2	-	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+		-	-
18	3	-	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
19	1	-	-	-	+	+	+	-	-	w+	-	-	+	-	+	-	-	+	-	-	-
19	2	-	-	-	+	+	+	+	w+	+	-	-	+	-	+	-	-	+	-	-	
19	3	-	-	-	+	+	+	+	w+	+	-	-	+	-	+	-	-	+	-		-
20	1	+	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	
20	2	+	-	-	+	+	+	+	-	+	-	-	+	-	+	-		+		-	-
20	3	+	-	-	+	+	+	+	-	+	-	-	+	-	+	-		+	-		-
21	1	-	-	-	+	+	+	-	-	w+	-	-	+	-	+	-	-	+			
21	2	+	-		+	+	+	+	-	+	-	-	+	-	+	-	-	+	-		-
21	3	+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	
22	1	-	-	-	-	+	w+	-	-	-	-	-	+	-	+	-	-	+		-	-
22	2	+	-	-	w+	+	w+	+	-	-	-	-	+	-	+	-	-	+		-	
22	3	+	-	-	w+	+	w+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
23	1	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+			-
23	2	+	-	-	w+	+	w+	+	w+	-	-	-	+	-	+		-	+		-	-
23	3	+	-	-	w+	+	w+	+	w+	-	-	-	+	-	+	-	-	+		-	
24	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
24	2	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
24	3	w+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+		-	-
25	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
25	2	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	
25	3	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
26	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	
26	2	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
26	3	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
27	1	-	-	-	+	+	+	-	-	w+	-	-	+	-	+	-	-	+	-	-	-
27	2	w+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	
27	3	+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-		-
28	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
28	2	+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
28	3	+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
29	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
29	2	-	-	-	w+	-	w+	-	-	w+	-		-	-	+	-	-	-	-	-	-
29	3	-	-	-	+	-	w+	-	-	w+	-	-	-	-	+	-	-	-	-	-	-
30	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
30	2	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
30	3	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
31	1	-	-	-	+	+	w+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
31	2	-	-	-	+	+	+	w+	-	-	-	-	+	-	+	-	-	+	-	-	-
31	3	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
32	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
32	2	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
32	3	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
33	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
33	2	+	-	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-
33	3	+	-	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-
34	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
34	2	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	+	+	-	-	-
34	3	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	+	+	-	-	-
35	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
35	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
35	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
36	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
36	2	-	-	-	+	+	+	+	w+	-	-	-	+	-	+	-	-	+	-	-	-
36	3	+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
37	1	-	w+	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
37	2	-	+	-	+	+	+	+	w+	+	-	-	+	-	+	-	-	+	-	-	-
37	3	+	+	-	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-	-	-
38	1	-	-	-	-	-	-	-	-	-	-	-	-	-	w+	-	-	w+	-	-	-
38	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
38	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
39	1	-	-	-	-	-	-	-	-	-	-	-	-	-	w+	-	-	w+	-	-	-
39	2	-	w+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
39	3	-	w+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
40	1	-	-	-	-	-	-	-	-	-	-	-	-	-	w+	-	-	-	-	-	-
40	2	-	w+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
40	3	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
41	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
41	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
41	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
42	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
42	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
42	3	w+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
43	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	
43	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	
43	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
44	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
44	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
44	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
45	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	
45	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		+	-	-	-
45	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
46	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	W+	-	-	-
46	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+			
46	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-		-
47	1	+	+	-	-	+	-	+	+	-	-	-	+	-	+	W+	W+	+		-	-
47	2	+	+	-	+	+	-	+	+	-	-	-	+	-	+	W+	+	+	W+	W+	-
47	3	+	+	-	+	+	-	+	+	-	-	-	+	-	+	W+	+	+	W+	W+	-
48	1	W+	-	-	W+	+	-	W+	W+	+	-	+	+	-	+	+	+	+		+	-
48	2	+	W+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+	-	+	
48	3	+	W+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+	-	+	-
49	1	-	-	-	-	+	-	+	+	-	-	-	+	-	+	+	+	+	-	-	-
49	2	+	W+	-	W+	+	-	+	+	-	-	-	+	-	+	+	+	+	-	-	-
49	3	+	W+	-	+	+	-	+	+	-	-	-	+	-	+	+	+	+	-	-	
50	1	+	-	-	W+	+	-	+	+	-	-	-	+	-	+	+	+	+	-	+	
50	2	+	W+	-	+	+	-	+	+	-	-	-	+	-	+	+	+	+		+	-
50	3	+	W+	-	+	+	-	+	+	-	-	-	+	-	+	+	+	+	-	+	-
51	1	+	+	-	W+	+	-	+	+	-	-	-	+	-	+	-	-	+		-	
51	2	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	W+	+			
51	3	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	W+	+	-	-	-
52	1	+	+	-	W+	+	-	+	+	-	-	-	+	-	+	-	-	+	-		
52	2	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	W+	+	-		
52	3	+	+	-	+	+	-	+	+	-	-	-	+	-	+	-	W+	+	-	-	-
53	1	-	-	-	+	+	+	-	W+	W+	-	-	+	-	+	+	+	+		+	
53	2	W+	-	-	+	+	+	+	W+	+	-	-	+	-	+	+	+	+	-	+	-
53	3	W+	-	-	+	+	+	+	W+	+	-	-	+	-	+	+	+	+	-	+	-
54	1	-	-	-	+	+	+	-	-	W+	-	-	+	-	+	-	-	+		-	
54	2	W+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
54	3	W+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
55	1	-	-	-	W+	W+	W+	-	W+	-	-	-	W+	-	W+	-	-	W+	-		-
55	2	W+	-	-	W+	W+	+	-	W+	-	-	-	+	-	W+	-	-	+	-	-	-
55	3	W+	-	-	W+	+	+	-	W+	-	-	-	+	-	+	-	-	+	-	-	-
56	1	-	-	W+	+	+	+	-	W+	W+	W+	-	+	-	+	+	+	+	-	+	-
56	2	-	-	W+	+	+	+	+	W+	W+	W+	-	+	-	+	+	+	+	-	+	-
56	3	-	-	W+	+	+	+	+	W+	W+	W+	-	+	-	+	+	+	+	-	+	

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
57	1	-	+	+	+	+	+	w+	+	+	+	-	+	-	+	+	+	+	-	+	-
57	2	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	-
57	3	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	-
58	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
58	2	w+	-	-	+	+	+	+	w+	w+	-	-	+	-	+	-	-	+	-	-	-
58	3	+	-	-	+	+	+	+	w+	w+	-	-	+	-	+	-	-	+	-	-	-
59	1	-	-	-	+	+	+	-	-	w+	-	-	+	-	+	-	-	+	-	-	-
59	2	-	-	-	+	+	+	+	-	+	-	-	+	-	+	w+	-	+	-	-	-
59	3	+	-	-	+	+	+	+	-	+	-	-	+	-	+	w+	-	+	-	-	-
60	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
60	2	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
60	3	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
61	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
61	2	-	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
61	3	w+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
62	1	-	-	-	+	+	+	-	-	w+	-	-	+	-	+	-	-	+	-	-	-
62	2	-	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
62	3	w+	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-
63	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
63	2	w+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
63	3	+	-	-	+	+	+	+	+	-	w+	-	+	-	+	-	-	+	-	-	-
64	1	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-
64	2	w+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
64	3	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-
65	1	-	-	-	+	+	+	w+	w+	-	-	-	+	-	+	-	-	+	-	-	-
65	2	w+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
65	3	+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-
66	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
66	2	w+	-	-	w+	w+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
66	3	w+	-	-	w+	w+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
67	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
67	2	-	-	-	w+	w+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
67	3	-	-	-	w+	w+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
68	1	-	-	-	w+	w+	w+	-	-	-	-	-	-	-	+	-	-	w+	-	-	-
68	2	-	-	-	+	+	w+	-	-	-	-	-	-	-	+	-	-	+	-	-	-
68	3	-	-	-	+	+	w+	-	-	-	-	-	-	-	+	-	-	+	-	-	-
69	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
69	2	-	-	-	w+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
69	3	-	-	-	w+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
70	1	-	-	-	-	w+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
70	2	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
70	3	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
71	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-		
71	2	-	w+	w+	-	-	w+	-	-	w+	-	-	-	-	+	-	-	+			
71	3	-	w+	w+	-	-	w+	-	-	w+	-	-	-	-	+		-	+			
72	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-				-
72	2	-	w+	+	-	w+	-	-	-	w+	-	-	-	-	+	+	-			w+	-
72	3	-	w+	+	-	w+	-	-	w+	w+	-	-	-	-	w+	w+	-	-		+	-
73	1	-	-	-	-	-	-	-	-	w+	-	-	-	-	+	-	-				
73	2	w+	w+	+	-	w+	w+	-	w+	w+	-	-	-	-	+	+	-	-		-	
73	3	w+	w+	+	-	w+	w+	-	w+	w+	-	-	-	-	+	+	-		-	-	
74	1	-	-	w+	-	-	w+	-	-	+	-	-	+	-	+	w+	w+	+			
74	2	-	w+	+	-	w+	+	-	w+	+	-	-	+	-	+	+	+	+			-
74	3	w+	w+	+	-	w+	+	-	w+	+	-	-	+	-	+	+	+	+			
75	1	-	-	-	-	-	w+	-	-	+	-	-	+	-	+	w+	w+	+	-		
75	2	-	+	+	w+	w+	+	-	w+	+	-	-	+	-	+	+	+	+		-	
75	3	w+	+	+	w+	w+	+	-	w+	+	-	-	+	-	+	+	+	+		-	
76	1	+	-	w+	w+	-	-	-	w+	-	w+	-	w+	-	+	w+	w+	+	+	+	w+
76	2	+	+	+	+	w+	w+	-	+	+	+	-	+	-	+	w+	w+	+	+	+	+
76	3	+	+	+	+	+	+	-	+	+	+	-	+	-	+	w+	w+	+	+	+	+
77	1	-	w+	w+	w+	-	w+	-	w+	+	w+	-	+	-	+	w+	w+	+	+	w+	
77	2	-	+	+	+	w+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+
77	3	w+	+	+	+	w+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+
78	1	-	-	-	-	-	-	-	-	-	-	-	+	-	+		-				
78	2	-	+	+	-	+	w+	-	-	-	-	-	+	-	+	-		w+	-	-	
78	3	-	+	+	-	+	w+	-	-	-	-	-	+	-	+	-	-	w+			
79	1	-	-	-	-	-	-	-	-	-	-	-	-		w+	-	-	-		-	
79	2	-	+	+	-	+	-	-	-	-	-	-	+	-	+	-		w+	-		-
79	3	-	+	+	-	+	-	-	-	-	-	-	+	-	+	-		w+		-	
80	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-		
80	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-		
80	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		-	-	-	
14048	1	+	-	+	-	+	+	-	-	-	+	-	+	-	+	+	+	+	-	-	+
14048	2	+	-	+	w+	+	+	-	-	-	+	-	+	-	+	+	+	+	-	-	+
14048	3	+	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+		-	+
14635	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14635	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
14635	3	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	
14715	1	-	-	-	+	-	+	-	-	-	+	-	+	-	+	-	-	+	-		-
14715	2	-	-	-	+	-	+	-	-	-	+	-	+		+	-	-	+	-		
14715	3	-	-	-	+	-	+		-	-	+	-	+	-	+	-	-	+		-	-
15338	1	+	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	+	+	-	-
15338	2	+	-	-	+	-	+	-	-	-	-	-	+	-	+	-	+	+	+		
15338	3	+	-	-	+	-	+	-	-	-	w+	-	+	-	+	-	+	+	+	-	

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
15468	1	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-
15468	2	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-
15468	3	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-
17749	1	-	-	+	-	+	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-
17749	2	-	-	+	-	+	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-
17749	3	-	-	+	-	+	+	-	-	-	+	-	+	-	+	-	w+	+	-	-	-
17802	1	-	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	-	-	-
17802	2	-	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	+	-	-
17802	3	-	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	+	-	-
19109	1	+	-	-	+	-	+	-	-	-	-	-	+	-	+	+	+	+	+	-	-
19109	2	+	-	-	+	-	+	-	-	-	-	-	+	-	+	+	+	+	+	-	-
19109	3	+	-	-	+	-	+	-	-	-	w+	-	+	-	+	+	+	+	+	-	-
19264	1	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-
19264	2	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-
19264	3	w+	-	+	+	-	+	w+	-	-	-	-	-	-	+	-	-	+	+	-	-
23211	1	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	-	+	-	-	-
23211	2	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	-	+	+	-	-
23211	3	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	-	+	+	-	-
2588	1	-	-	+	+	-	+	w+	-	+	+	+	+	-	+	+	+	+	+	-	-
2588	2	-	-	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-
2588	3	w+	-	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-
25914	1	-	-	-	-	-	+	-	-	-	-	-	-	-	+		-	+	-	-	-
25914	2	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-
25914	3	w+	-	-	w+	-	+	w+	-	-	-	-	-	-	+	-	-	+	-	-	-
25916	1	-	-	-	-	-	+	-	-	-	-	-	w+	-	+	-	-	-	-	-	-
25916	2	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-
25916	3	w+	-	-	w+	-	+	w+	-	-	-	-	+	-	+	-	-	-	-	-	-
25917	1	+	-	-	+	-	+	-	-	-	-	-	w+	-	+	+	+	+	+	-	-
25917	2	+	-	+	+	-	+	-	w+	-	-	-	+	-	+	+	+	+	+	-	-
25917	3	+	-	+	+	-	+	w+	+	-	-	-	+	-	+	+	+	+	+	-	-
25919	1	+	w+	+	+	-	+	-	-	+	-	+	w+	-	+	+	+	+	+	-	-
25919	2	+	w+	+	+	-	+	-	w+	+	-	+	+	-	+	+	+	+	+	-	-
25919	3	+	+	+	+	-	+	w+	+	+	-	+	+	-	+	+	+	+	+	-	-
25920	1	-	-	-	-	-	+	-	-	-	-	-	-	-	w+	-	-	+	-	-	-
25920	2	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-
25920	3	w+	-	-	w+	-	+	w+	-	-	-	-	-	-	+	-	-	+	-	-	-
27013	1	-	-	-	-	-	-	w+	-	-	-	-	-	-	-	-	-	-	-	-	-
27013	2	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
27013	3	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
27043	1	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-
27043	2	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-
27043	3	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	-	+	+	+	-

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
27562	1	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
27562	2	+	-	-	-	+	+	-	-	-	-	-	w+	-	+	-	-	-	-	-	-
27562	3	+	-	-	w+	+	+	w+	-	-	-	-	+	-	+	-	-	-	-	-	-
29988	1	-	+	+	+	-	+	-	-	+	+	-	-	-	w+	-	-	+	-	-	-
29988	2	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	-
29988	3	-	+	+	+	-	+	w+	-	+	+	-	w+	-	+	-	-	+	-	-	-
33125	1	-	-	+	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-
33125	2	+	-	+	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-
33125	3	+	w+	+	-	-	-	w+	-	-	-	-	+	-	+	+	+	-	-	-	-
33466	1	+	+	+	-	+	+	+	-	-	+	+	+	-	+	+	+	+	-	-	-
33466	2	+	+	+	w+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-
33466	3	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-
33509	1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
33509	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
33509	3	+	w+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
33564	1	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+	+	w+	-	-
33564	2	+	-	+	+	-	+	-	-	-	-	-	+	-	+	+	+	+	+	-	-
33564	3	+	w+	+	+	-	+	-	-	-	w+	-	+	-	+	+	+	+	+	-	-
33653	1	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-
33653	2	+	-	w+	+	+	+	-	-	-	-	-	w+	-	+	+	+	+	-	-	-
33653	3	+	w+	+	+	+	+	-	-	-	w+	-	+	-	+	+	+	+	-	-	-
33809	1	+	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
33809	2	+	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
33809	3	+	w+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
33934	1	+	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-
33934	2	+	-	-	+	-	+	-	-	-	-	-	w+	-	+	+	+	+	-	-	-
33934	3	+	w+	w+	+	-	+	-	-	w+	w+	-	+	-	+	+	+	+	-	-	-
35016	1	+	-	+	+	+	+	-	-	-	-	+	+	-	+	+	+	+	-	-	+
35016	2	+	-	+	+	+	+	-	-	-	-	+	+	-	+	+	+	+	-	-	+
35016	3	+	w+	+	+	+	+	-	-	w+	w+	+	+	-	+	+	+	+	-	-	+
35048	1	+	-	+	+	+	+	-	-	+	-	-	-	-	+	+	+	+	w+	-	-
35048	2	+	+	+	+	+	+	+	-	+	+	+	w+	-	+	+	+	+	+	-	-
35048	3	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	w+	-
35084	1	-	+	+	w+	+	+	-	-	-	+	-	w+	-	+	-	-	+	-	+	-
35084	2	-	+	+	w+	+	+	-	-	-	+	-	+	-	+	-	-	+	-	+	-
35084	3	w+	+	+	w+	+	+	-	-	+	+	-	+	-	+	-	+	+	-	+	-
35912	1	-	+	+	+	-	+	-	-	-	+	-	w+	-	+	-	-	+	-	+	-
35912	2	-	+	+	+	+	+	-	-	-	+	-	+	-	+	-	-	+	-	+	-
35912	3	w+	+	+	+	+	+	-	-	+	+	-	+	-	+	-	-	+	-	+	-
43341	1	-	+	-	w+	+	+	+	-	-	+	-	w+	-	+	-	-	+	+	+	-
43341	2	-	+	-	w+	+	+	+	w+	-	+	-	+	-	+	-	-	+	+	+	-
43341	3	w+	+	+	w+	+	+	+	+	w+	+	-	+	-	+	-	-	+	+	+	-

Appendix D continued:

Strain	Wk	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
43979	1	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
43979	2	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
43979	3	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
7744	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7744	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7744	3	-	w+	-	-	-	-	-	w+	-	-	-	-	-	-	-	-	-	-	w+	w+
9031	1	-	-	+	-	-	+	-	-	+	+	+	+	-	+	+	+	+	-	-	-
9031	2	-	-	+	-	-	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-
9031	3	w+	-	+	-	-	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-
9067	1	-	-	-	+	-	+	-	-	-	+		-	-	+	-	-	-	-	-	-
9067	2	-	-	-	+	-	+	-	-	-	+	-	-	-	+	+	+	w+	+	-	-
9067	3	w+	-	-	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+	w+	-
9578	1	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-
9578	2	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	+	-	+	-
9578	3	w+	-	-	+	-	+	-	-	-	w+	-	+	-	+	-	-	+	-	+	-

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
1	1	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	+
1	2	-	+	-	-	-	-	-	-	+	+	+	w+	+	-	-	+	-	-	-	+
1	3	-	+	-	-	-	-	w+	-	+	+	+	+	+	+	-	+	-	-	-	+
2	1	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	+
2	2	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
2	3	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	w+	+
3	1	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	+
3	2	-	+	-	-	-	-	-	-	+	+	+	w+	+	-	-	+	-	-	-	+
3	3	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+
4	1	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	w+	-	-	-	+
4	2	-	+	-	-	-	-	w+	w+	+	+	+	+	+	-	+	+	-	-	-	+
4	3	w+	+	w+	w+	w+	-	w+	w+	+	+	+	+	+	w+	+	+	w+	-	+	+
5	1	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	w+	-	-	-	+
5	2	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
5	3	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+
6	1	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	w+	-	-	-	+
6	2	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
6	3	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
7	1	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	w+	-	-	-	-
7	2	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
7	3	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	w+	-
8	1	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	w+	-	-	-	+
8	2	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
8	3	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
9	1	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	w+
9	2	-	+	-	-	-	-	-	-	+	+	+	w+	+	-	-	+	-	-	-	w+
9	3	-	+	-	-	-	-	-	-	+	+	+	w+	+	-	-	+	-	-	-	+
10	1	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-
10	2	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
10	3	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
11	1	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	-	-	-	-
11	2	-	+	-	-	-	-	+	+	+	+	+	w+	-	-	-	+	-	-	-	-
11	3	w+	+	-	-	-	-	+	+	+	+	+	w+	-	-	-	+	-	-	-	-
12	1	-	-	-	-	-	-	w+	-	+	+	+	-	-	-	-	+	-	-	-	w+
12	2	+	+	+	+	+	-	+	-	+	+	+	-	w+	-	-	+	-	-	-	w+
12	3	+	+	+	+	+	-	+	w+	+	+	+	-	w+	-	-	+	-	-	-	+
13	1	-	w+	-	-	-	-	w+	+	+	+	+	-	-	-	-	+	-	-	-	-
13	2	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-
13	3	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-
14	1	-	w+	-	-	-	-	w+	-	+	+	+	-	-	-	-	+	-	-	-	w+
14	2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	w+
14	3	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
15	1	-	-	-	-	-	-	w+	w+	+	+	+	-	-	-	-	+	-	-	-	w+
15	2	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	-	-	-	w+
15	3	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	-	-	-	+
16	1	w+	+	w+	w+	w+	w+	+	+	+	+	+	-	-	w+	w+	+	w+	-	+	+
16	2	+	+	+	+	+	+	+	+	+	+	+	+	+	w+	+	+	+	+	+	+
16	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	1	-	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	+
17	2	+	+	+	+	+	-	+	+	+	+	+	-	-	+	-	+	-	-	+	+
17	3	+	+	+	+	+	w+	+	+	+	+	+	-	-	+	-	+	-	-	+	+
18	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
18	2	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
18	3	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
19	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
19	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
19	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
20	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
20	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
20	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
21	1	+	+	-	-	-	-	+	+	-	+	+	-	-	+	-	+	-	-	+	+
21	2	+	+	-	-	-	-	+	+	-	+	+	-	-	+	-	+	-	-	+	+
21	3	+	+	-	-	-	-	+	+	-	+	+	+	w+	+	-	+	-	-	+	+
22	1	+	+	-	-	-	-	+	w+	-	-	+	-	-	+	-	+	-	-	+	+
22	2	+	+	-	-	-	-	+	w+	-	-	+	-	-	+	-	+	-	-	+	+
22	3	+	+	-	-	-	-	+	w+	-	-	+	-	-	+	-	+	-	-	+	+
23	1	+	+	-	-	-	-	+	w+	-	-	-	-	-	+	-	+	-	-	+	+
23	2	+	+	-	-	-	-	+	w+	-	-	-	-	-	+	-	+	-	-	+	+
23	3	+	+	-	-	-	-	+	w+	-	-	-	-	-	+	-	+	-	-	+	+
24	1	+	+	-	-	-	-	+	w+	-	-	-	-	-	+	-	+	-	-	+	+
24	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
24	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
25	1	+	+	-	-	-	-	+	w+	-	-	+	-	-	+	-	+	-	-	+	+
25	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
25	3	+	+	-	-	-	-	+	+	-	-	+	+	w+	+	-	+	-	-	+	+
26	1	+	+	-	-	-	-	+	+	+	-	+	-	-	+	-	+	-	-	+	+
26	2	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	+
26	3	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	+
27	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
27	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
27	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
28	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
28	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
28	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
29	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	2	-	-	-	-	-	-	-	+	-	-	w+	-	-	-	-	-	-	-	-	-
29	3	-	-	-	-	-	-	-	+	-	-	w+	-	-	-	-	-	-	-	-	-
30	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
30	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
30	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
31	1	w+	+	-	-	-	-	+	+	-	-	w+	-	-	+	-	+	-	-	+	+
31	2	+	+	-	-	-	-	+	+	-	-	w+	-	-	+	-	+	-	-	+	+
31	3	+	+	-	-	-	-	+	+	-	-	w+	-	-	+	-	+	-	-	+	+
32	1	w+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
32	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
32	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
33	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
33	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
33	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+
34	1	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-
34	2	+	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-
34	3	+	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-
35	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	w+	-	-	+	+
35	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+
35	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+
36	1	-	+	-	-	-	-	+	w+	-	w+	w+	-	-	+	-	w+	-	-	+	+
36	2	w+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
36	3	w+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
37	1	w+	+	-	-	-	-	+	w+	-	w+	+	-	-	+	-	w+	-	-	+	+
37	2	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
37	3	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
38	1	-	-	-	-	-	-	w+	-	-	-	w+	-	-	-	-	-	-	-	-	
38	2	-	-	-	-	-	-	+	w+	+	+	+	-	-	+	-	-	-	-	-	-
38	3	-	-	-	-	-	-	+	w+	+	+	+	-	-	+	-	w+	-	-	-	-
39	1	-	-	-	-	-	-	w+	-	-	-	w+	-	-	-	-	-	-	-	-	-
39	2	-	-	-	-	-	-	+	w+	+	+	+	-	-	+	-	-	-	-	-	-
39	3	-	-	-	-	-	-	+	w+	+	+	+	-	-	+	-	w+	-	-	-	-
40	1	-	-	-	-	-	-	w+	-	-	-	w+	-	-	-	-	-	-	-	-	-
40	2	-	-	-	-	-	-	+	w+	+	+	+	-	-	+	-	-	-	-	-	-
40	3	-	-	-	-	-	-	+	w+	+	+	+	-	-	+	-	w+	-	-	-	-
41	1	-	-	-	-	-	-	+	-	+	w+	+	-	-	w+	-	w+	-	-	-	-
41	2	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	-
41	3	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	-
42	1	-	-	-	-	-	-	+	-	+	w+	+	-	-	+	-	w+	-	-	-	-
42	2	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	-
42	3	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	-

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
43	1	-	-	-	-	-	-	+	-	+	w+	+	-	-	+	-	w+	-	-	-	-
43	2	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	-
43	3	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	w+	-	-
44	1	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	-	-	-	-	-
44	2	-	-	-	-	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-
44	3	-	-	-	-	-	-	+	-	+	+	+	+	w+	+	-	w+	-	-	-	-
45	1	-	-	-	-	-	-	w+	-	+	-	w+	-	-	w+	-	-	-		-	-
45	2	-	-	-	-	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-
45	3	-	-	-	-	-	-	+	-	+	+	+	+	w+	+	-	w+	-	-	-	-
46	1	-	-	-	-	-	-	-	-	+	-	-	w+	-	+	w+	w+	w+	-	+	+
46	2	-	-	-	-	-	-	+	-	+	+	+	+	+	+	w+	w+	+	-	+	+
46	3	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	w+	+	-	+	+
47	1	-	+	-	-	-	-	-	-	+	+	+	w+	-	-	-	+	-	-	-	+
47	2	w+	+	w+	-	-	-	+	w+	+	+	+	+	+	-	-	+	-	-	-	+
47	3	w+	+	w+	-	-	-	+	+	+	+	+	+	+	-	-	+	-	w+	w+	+
48	1	-	+	+	+	+	-	w+	+	+	+	w+	+	+	+	+	+	+	-	+	+
48	2	-	+	+	+	+	w+	w+	+	+	+	+	+	+	+	+	+	+	-	+	+
48	3	-	+	+	+	+	w+	w+	+	+	+	+	+	+	+	+	+	+	-	+	+
49	1	+	+	-	-	-	-	-	-	+	w+	+	w+	w+	+	w+	+	-	-	.	+
49	2	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+
49	3	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	w+	w+	+
50	1	-	+	-	+	+	-	w+	+	+	+	+	+	-	+	+	+	+	-	+	+
50	2	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
50	3	-	+	-	+	+	w+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
51	1	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	+
51	2	-	w+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
51	3	-	w+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
52	1	-	w+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	+
52	2	-	w+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+
52	3	-	w+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	w+	-	+
53	1	-	+	-	+	+	-	+	+	+	+	+	+	w+	+	+	+	+	-	+	+
53	2	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
53	3	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
54	1	w+	+	-	-	-	-	+	+	w+	+	+	-	-	+	-	+	-	-	+	+
54	2	+	+	-	-	-	-	+	+	w+	+	+	-	-	+	-	+	-	-	+	+
54	3	+	+	-	-	-	-	+	+	w+	+	+	-	-	+	-	+	-	-	+	+
55	1	-	-	-	-	-	-	w+	w+	w+	w+	w+	-	-	-	-	-	-	-	-	-
55	2	-	-	-	-	-	-	w+	+	w+	+	w+	-	-	-	-	-	-	-	+	-
55	3	-	-	-	-	-	-	w+	+	w+	+	w+	-	-	-	-	w+	-	-	+	+
56	1	-	+	w+	+	+	-	+	+	+	+	+	+	w+	+	+	+	+	-	+	+
56	2	w+	+	w+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
56	3	w+	+	w+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
57	1	w+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
57	2	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
57	3	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
58	1	-	+	-	-	-	-	+	w+	-	w+	w+	-	-	+	-	+	-	-	+	+
58	2	+	+	-	-	-	-	+	w+	-	w+	w+	-	-	+	-	+	-	-	+	+
58	3	+	+	-	-	-	-	+	+	-	w+	w+	-	-	+	-	+	-	-	+	+
59	1	-	+	-	-	-	-	+	w+	-	w+	+	-	-	+	-	w+	-	-	+	w+
59	2	+	+	-	-	-	-	+	w+	-	w+	+	-	-	+	-	+	-	-	+	+
59	3	+	+	-	-	-	-	+	w+		w+	+	-	-	+	-	+	-	-	+	+
60	1	w+	+	-	-	-	-	+	w+	-	w+	+	-	-	+	-	+	-	-	+	+
60	2	+	+	-	-	-	-	+	w+	-	w+	+	-	-	+	-	+	-	-	+	+
60	3	+	+	-	-	-	-	+	w+	-	w+	+	-	-	+	-	+	-	-	+	+
61	1	w+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
61	2	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
61	3	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	+	-	-	+	+
62	1	-	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	-	-	-	+	+
62	2	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	w+	-	-	+	+
62	3	+	+	-	-	-	-	+	+	-	w+	+	-	-	+	-	w+	-	-	+	+
63	1	-	+	-	-	-	-	+	+	+	w+	+	-	-	+	-	-	-	-	+	+
63	2	+	+	-	-	-	-	+	+	+	w+	+	-	-	+	-	-	-	-	+	+
63	3	+	+	-	-	-	-	+	+	+	w+	+	-	-	+	-	w+	-	-	+	+
64	1	w+	+	-	-	-	-	+	+	w+	w+	+	-	-	+	-	-	-	-	+	+
64	2	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-	+	+
64	3	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	w+	-	-	+	+
65	1	-	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	+	+
65	2	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	+	+
65	3	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	w+	-	-	+	+
66	1	-	-	-	-	-	-	+	-	w+	w+	w+	-	-	-	-	-	-	-	-	-
66	2	-	w+	-	-	-	-	+	w+	+	+	+	-	-	-	-	-	-	-	-	-
66	3	-	w+	-	-	-	-	+	+	+	+	+	-	-	+	-	w+	-	-	w+	-
67	1	-	-	-	-	-	-	+	w+	+	w+	+	-	-	+	-	-	-		-	-
67	2	-	w+	-	-	-	-	+	w+	+	+	+	+	-	+	-	-	-	-	-	
67	3	-	w+	-	-	-	-	+	+	+	+	+	+	+	+	-	w+	-	w+	-	-
68	1	w+	w+	-	-	-	-	w+	w+	w+	w+	-	-	-	+	-	-	-	-	-	-
68	2	+	+	-	-	-	-	w+	+	+	w+	w+	+	-	+	-	-	+	-	-	-
68	3	+	+	-	-	-	-	w+	+	+	+	+	+	w+	+	w+	w+	+	-	-	-
69	1	-	-	-	-	-	-	+	-	+	w+	+	-	-	+	-	-	-	-	-	-
69	2	-	w+	-	-	-	-	+	w+	+	+	+	+	+	+	-	-	-	-	-	-
69	3	-	w+	-	-	-	-	+	w+	+	+	+	+	+	+	-	w+	-	w+	-	-
70	1	-	-	-	-	-	-	+	-	+	w+	+	-	-	+	-	-	-	-	-	-
70	2	-	-	-	-	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-
70	3	-	-	-	-	-	-	+	-	+	+	+	+	-	+	-	w+	-	-	-	-

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
71	1	-	-	-	-	-	-	w+	-	+	w+	+	-	-	+	-	+	-	-	-	-
71	2	-	-	-	-	-	-	+	-	+	w+	+	+	-	+	-	+	+	-	-	-
71	3	-	-	-	-	-	-	+	-	+	w+	+	+	-	+	+	+	+	-	w+	+
72	1	w+	+	-	-	-	-	-	-	w+	-	-	w+	-	+	w+	+	w+	-	-	-
72	2	+	+	-	-	-	-	w+	w+	+	-	+	+	+	+	+	+	+	-	-	-
72	3	+	+	-	-	-	w+	w+	w+	+	-	+	+	+	+	+	+	+	w+	-	-
73	1	w+	+	-	-	-	-	-	-	w+	-	-	-	-	+	w+	+	w+	-	-	w+
73	2	+	+	-	-	-	+	w+	w+	+	-	+	+	+	+	+	+	+	-	-	w+
73	3	+	+	-	-	-	+	w+	w+	+	-	+	+	+	+	+	+	+	w+	-	+
74	1	-	+	-	-	w+	-	-	-	+	+	+	-	+	+	w+	+	w+	-	+	+
74	2	+	+	-	-	+	w+	-	+	+	+	+	+	+	+	+	+	+	-	+	+
74	3	+	+	-	-	+	w+	w+	+	+	+	+	+	+	+	+	+	+	w+	+	+
75	1	-	+	-	-	w+	-	-	-	+	+	+	-	w+	+	+	+	w+	-	+	+
75	2	+	+	-	w+	+	w+	+	+	+	+	+	+	+	+	+	+	+	w+	+	+
75	3	+	+	-	w+	+	w+	+	+	+	+	+	+	+	+	+	+	+	w+	+	+
76	1	w+	+	w+	-	-	-	w+	-	+	+	+	-	w+	+	w+	+	w+	-	+	+
76	2	+	+	+	w+	+	w+	+	+	+	+	+	+	+	+	w+	+	w+	-	+	+
76	3	+	+	+	w+	+	w+	+	+	+	+	+	+	+	+	w+	+	w+	w+	+	+
77	1	w+	+	w+	-	w+	-	w+	-	+	+	+	-	w+	+	+	+	w+	-	+	+
77	2	+	+	+	w+	+	w+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
77	3	+	+	+	w+	+	w+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
78	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	w+	+	-	-	w+	+
78	2	-	w+	-	-	-	-	+	-	+	w+	+	+	w+	+	+	+	+	-	+	+
78	3	-	w+	-	-	-	-	+	-	+	w+	+	+	w+	+	+	+	+	-	+	+
79	1	-	-	-	-	-	-	w+	-	w+	-	-	-	-	+	-	-	-	-	-	w+
79	2	-	-	-	-	-	-	+	-	+	w+	+	+	-	+	-	+	+	-	-	w+
79	3	w+	-	-	-	-	-	+	-	+	w+	+	+	w+	+	w+	+	+	-	w+	+
80	1	-	-	-	-	-	-	+	-	+	-	w+	-	-	+	-	+	-	-	+	w+
80	2	-	-	-	-	-	-	+	-	+	w+	+	+	-	+	-	+	-	-	+	w+
80	3	-	-	-	-	-	-	+	-	+	w+	+	+	-	+	-	+	+	-	+	w+
14048	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
14048	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
14048	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	w+	+	+	-
14635	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14635	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14635	3	-	-	-	-	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14715	1	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-
14715	2	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
14715	3	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
15338	1	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	-
15338	2	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	+
15338	3	+	+	-	-	w+	-	+	+	+	+	+	-	+	+	-	+	-	-	+	+

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
15468	1	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-
15468	2	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-
15468	3	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-
17749	1	w+	-	+	-	+	-	+	+	+	+	+	-	-	-	-	+	-	-	+	-
17749	2	+	+	+	-	+	+	+	+	+	+	+	-	+	-	-	+	w+	-	+	-
17749	3	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-
17802	1	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	-
17802	2	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	-
17802	3	+	+	-	-	w+	-	+	+	+	+	+	-	+	+	-	+	-	-	+	-
19109	1	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	-
19109	2	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	+
19109	3	+	+	-	-	w+	-	+	+	+	+	+	-	+	+	-	+	-	-	+	+
19264	1	-	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	-
19264	2	-	+	-	-	-	-	+	+	+	+	+	-	w+	+	-	+	-	-	+	
19264	3	-	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	-
23211	1	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	-
23211	2	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-
23211	3	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-
2588	1	+	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-
2588	2	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
2588	3	+	+	w+	w+	+	w+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
25914	1	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-
25914	2	-	+	-	-	-	-	+	+	w+	w+	+	-	-	+	-	+	-	-	+	-
25914	3	-	+	-	-	-	-	+	+	w+	+	+	-	w+	+	-	+	-	-	+	-
25916	1	+	+	-	-	-	-	+	+	w+	-	-	+	+	+	-	+	-	-	+	-
25916	2	+	+	-	-	-	-	+	+	+	+	w+	+	+	+	+	+	+	+	+	-
25916	3	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
25917	1	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-	-	+	-
25917	2	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25917	3	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25919	1	+	+	-	-	w+	-	+	+	+	+	+	+	+	+	+	+	w+	+	+	-
25919	2	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
25919	3	+	+	-	w+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
25920	1	-	+	-	-	-	-	+	+	-	-	+	+	+	+	-	+	-	-	+	
25920	2	-	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	w+	+	+	-
25920	3	-	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-
27013	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
27013	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
27013	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
27043	1	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	w+	-	-	+	-
27043	2	+	+	-	-	-	-	+	+	-	-	+	-	+	+	+	w+	-	-	+	-
27043	3	+	+	-	-	-	-	+	+	-	-	+	-	+	+	+	w+	-	-	+	-

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
27562	1	-	+	-	-	-	-	+	+	-	-	+	-	-	+	-	w+	-	-	+	-
27562	2	w+	+	-	-	-	-	+	+	-	w+	+	-	w+	+	-	w+	-	-	+	-
27562	3	+	+	-	-	-	-	+	+	-	+	+	-	+	+	-	w+	-	-	+	-
29988	1	-	+	-	w+	w+	-	+	+	+	+	+	-	-	+	-	-	-	-	+	-
29988	2	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	w+	-	-	+	-
29988	3	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	w+	-	-	+	-
33125	1	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
33125	2	+	+	-	-	-	-	+	-	+	-	w+	-	-	-	-	-	-	-	-	-
33125	3	+	+	-	-	-	-	+	-	+	-	w+	-	-	-	-	-	-	-	-	-
33466	1	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-
33466	2	+	+	w+	-	-	-	+	+	+	+	+	+	+	+	+	+	w+	-	+	-
33466	3	+	+	w+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
33509	1	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
33509	2	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
33509	3	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
33564	1	-	+	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	+	-
33564	2	-	+	-	-	-	-	+	-	+	+	+	-	+	+	-	+	-	-	+	-
33564	3	-	+	-	-	-	-	+	-	+	+	+	-	+	+	-	+	-	-	+	-
33653	1	-	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	-
33653	2	+	+	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	+	-
33653	3	+	+	-	-	-	-	+	+	+	+	+	-	w+	+	-	+	-	-	+	-
33809	1	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+
33809	2	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+
33809	3	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+
33934	1	-	+	-	-	-	-	+	-	+	+	+	-	-	+	+	+	-	-	+	-
33934	2	-	+	-	-	-	-	+	-	+	+	+	-	w+	+	+	+	-	-	+	-
33934	3	-	+	w+	-	-	w+	+	-	+	+	+	-	+	+	+	+	-	-	+	-
35016	1	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+
35016	2	+	+	-	-	-	-	+	+	+	+	+	w+	+	+	+	+	-	-	+	+
35016	3	+	+	w+	-	-	w+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
35048	1	+	+	-	-	-	-	+	w+	-	+	+	-	-	+	-	-	-	-	+	-
35048	2	+	+	-	-	-	+	+	+	-	+	+	-	w+	+	-	-	-	-	+	-
35048	3	+	+	w+	-	-	+	+	+	-	+	+	-	+	+	-	-	-	-	+	-
35084	1	-	+	-	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	+	-
35084	2	w+	+	-	-	-	-	+	w+	-	-	+	-	+	+	-	+	-	-	+	-
35084	3	+	+	-	-	-	-	+	+	-	-	+	-	+	+	-	+	-	-	+	-
35912	1	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-	+	-
35912	2	-	+	-		-	-	+	w+	-	-	-	-	+	+	-	+	-	-	+	-
35912	3	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	+	-	-	+	-
43341	1	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	+	-
43341	2	+	+	-	-	-	-	+	w+	-	-	+	-	+	+	-	+	-	-	+	-
43341	3	+	+	-	-	-	-	+	+	-	-	+	-	+	+	w+	+	+	-	+	-

Appendix D continued:

Strain	Wk	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
43979	1	+	+	-	-	-	-	-	-	+	-	-	+	+	+	+	+	-	-	-	-
43979	2	+	+	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	-
43979	3	+	+	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	-
7744	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+		+	-	-		-
7744	2	-	-	-	-	-	-	-	-	-	-	-	+	+	+	w+	+	-	-	-	-
7744	3	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	w+	w+	-
9031	1	+	+	-	-	+	-	w+	+	+	+	+	+	+	+	+	+	-	+	+	-
9031	2	+	+	+	-	+	-	w+	+	+	+	+	+	+	+	+	+	+	+	+	-
9031	3	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
9067	1	-	-	-	-	-	-	w+	+	+	+	+	-	-	-	-	-	-	-		
9067	2	-	-	-	-	-	-	w+	+	+	+	+	+	w+	-	-	-	-	-	+	-
9067	3	w+	-	-	-	w+	-	+	+	+	+	+	+	+	+	-	-	-	-	+	-
9578	1	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-	-	+	-
9578	2	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-
9578	3	+	+	-	-	w+	-	+	+	+	+	+	+	+	+	+	+	w+	+	+	-

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
1	1	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	+
1	2	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	+
1	3	+	-	+	-	+	-	-	+	-	-	-	-	-	-	+
2	1	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	w+
2	2	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	+
2	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
3	1	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
3	2	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	+
3	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
4	1	w+	-	-	-	-	-	-	w+	-	-	-	-	-	-	-
4	2	w+	-	-	-	-	-	-	w+	-	-	-	-	-	-	+
4	3	+	+	-	+	+	-	w+	+	-	-	w+	w+	w+	w+	+
5	1	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	w+
5	2	w+	-	-	-	-	-	-	+	-	-	-	-	-	-	+
5	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
6	1	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	2	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
6	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
7	1	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	w+
8	1	w+	-	-	-	-	-	-	w+	-	-	-	-	-	-	w+
8	2	w+	-	-	-	-	-	-	w+	-	-	-	-	-	-	+
8	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
9	1	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	2	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	2	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	3	+	-	-	-	w+	-	-	-	-	-	-	-	-	-	-
12	1	-	-	-	-	-	-	-	w+	-	-	-	-	-	-	-
12	2	-	-	-	-	w+	-	-	w+	-	-	-	-	-	-	+
12	3	+	-	-	-	w+	-	-	+	-	-	-	-	-	-	+
13	1	-	-	-	-	-	-	-	w+	-	-	-	-	-	-	-
13	2	-	-	-	-	-	-	-	w+	-	-	-	-	-	-	+
13	3	+	-	-	-	w+	-	-	+	-	-	-	-	-	-	+
14	1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	w+
14	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
14	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
15	1	-	-	-	-	-	-	-	w+	-	-	-	-	-	-	-
15	2	-	-	-	-	-	-	-	w+	-	-	-	-	-	-	+
15	3	+	-	-	-	w+	-	-	+	-	-	-	-	-	-	+
16	1	+	+	-	-	+	-	-	w+	-	-	+	w+	w+	+	-
16	2	+	+	-	-	+	-	-	w+	-	-	+	w+	w+	+	+
16	3	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+
17	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
17	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
17	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
18	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
18	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
18	3	+	-	-	-	+	-	w+	+	-	-	-	-	-	-	-
19	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
19	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
19	3	+	-	-	-	+	-	w+	+	-	-	-	-	w+	-	-
20	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
20	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
20	3	+	-	-	-	+	-	w+	+	-	-	-	-	-	-	-
21	1	+	-	-	-	+	-	-	-	-	-	-	-	-		-
21	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
21	3	+	-	-	-	+	-	w+	+	-	-	-	-	-	+	-
22	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
22	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
22	3	+	-	-	-	+	-	w+	+	-	-	-	-	-	-	-
23	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
23	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
23	3	+	-	-	-	+	-	w+	+	-	-	-	-	-	-	-
24	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
24	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
24	3	+	-	-	-	+	-	w+	-	-	-	-	-	-	-	-
25	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
25	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
25	3	+	-	-	-	+	-	w+	+	-	-	-	-	-	+	
26	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	
26	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
26	3	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
27	1	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
27	2	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
27	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
28	1	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
28	2	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
28	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
29	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	3	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	1	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
30	2	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
30	3	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-
31	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
31	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
31	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
32	1	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
32	2	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
32	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
33	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
33	2	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-
33	3	+	-	-	-	+	+	-	w+	-	-	-	-	-	-	-
34	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	3	w+	-	-	-	w+	-	-	-	-	-	-	-	-	-	-
35	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
35	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
35	3	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
36	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
36	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
36	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
37	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
37	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
37	3	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
38	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	3	w+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
39	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	3	w+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
40	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	3	w+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
41	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	3	w+	-	-	-	w+	-	-	w+	-	-	-	-	-	-	-
42	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	3	w+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
43	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	3	w+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
44	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
45	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	3	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
46	1	-	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
46	2	-	-	-	-	w+	-	-	+	+	-	-	-	-	-	-
46	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
47	1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
47	2	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+
47	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
48	1	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+
48	2	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+
48	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
49	1	-	-	-	-	+	-	-	w+	-	-	-	+	-	-	-
49	2	-	-	-	-	+	-	-	w+	-	-	-	+	-	-	+
49	3	-	-	-	-	+	-	-	+	w+	-	-	+	-	-	+
50	1	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+
50	2	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+
50	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
51	1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
51	2	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+
51	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
52	1	-	-	-	-	-	-	-	w+	-	-	-	-	-	-	w+
52	2	-	-	-	-	+	-	-	w+	+	-	-	-	-	-	+
52	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
53	1	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
53	2	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
53	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
54	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
54	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
54	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
55	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	2	-	-	-	-	w+	-	-	-	-	-	-	-	-	-	-
55	3	+	-	-	-	w+	-	-	-	-	-	-	-	-	-	-
56	1	w+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
56	2	w+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
56	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
57	1	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
57	2	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
57	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
58	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
58	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
58	3	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
59	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
59	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
59	3	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
60	1	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
60	2	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
60	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
61	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
61	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
61	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
62	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
62	2	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
62	3	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
63	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
63	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
63	3	+	-	-	-	+	w+	-	+	-	-	-	-	-	-	-
64	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
64	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
64	3	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
65	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
65	2	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
65	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
66	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	3	+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
67	1	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
67	2	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
67	3	+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
68	1	-	-	-	-	w+	-	-	-	-	-	-	-	-	-	-
68	2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
68	3	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
69	1	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
69	2	w+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
69	3	+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-
70	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	3	+	-	-	-	w+	-	-	+	-	-	-	-	-	-	-

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
71	1	-	-	-	-	-	-	w+	+	-	-	-	-	-	-	-
71	2	-	-	-	-	-	-	w+	+	-	-	-	-	-	-	-
71	3	w+	-	-	-	w+	-	w+	+	-	-	-	-	-	-	-
72	1	-	-	+	-	w+	-	-	-	-	-	-	w+	-	-	-
72	2	-	-	+	-	w+	-	-	-	-	-	-	w+	-	-	-
72	3	-	-	+	-	w+	-	w+	:	-	-	-	+	-	-	-
73	1	-	-	+	-	-	-	-	-	-	-	-	w+	-	-	-
73	2	-	-	+	-	-	-	-	-	-	-	-	w+	-	-	-
73	3	-	-	+	-	w+	-	-	w+	-	-	-	+	-	-	-
74	1	-	-	-	-	+	-	w+	+	-	-	-	+	-	-	+
74	2	-	-	-	-	+	-	w+	+	-	-	-	+	-	-	+
74	3	w+	-	-	-	+	-	w+	+	-	-	-	+	-	-	+
75	1	-	-	-	-	+	-	w+	+	-	-	-	+	-	-	w+
75	2	-	-	-	-	+	-	w+	+	-	-	-	+	-	-	+
75	3	w+	-	+	-	+	-	w+	+	-	-	-	+	-	-	+
76	1	w+	-	-	-	+	-	w+	+	-	-	-	-	-	+	-
76	2	w+	-	-	-	+	-	w+	+	-	-	-	-	-	+	-
76	3	+	-	-	-	+	-	w+	+	-	-	-	+	-	+	-
77	1	-	-	-	-	+	-	w+	+	-	-	-	+	-	-	-
77	2	-	-	-	-	+	-	w+	+	-	-	-	+	-	-	+
77	3	-	-	-	-	+	-	w+	+	-	-	+	+	-	-	+
78	1	w+	-	w+	-	+	-	w+	-	-	-	-	+	-	-	-
78	2	w+	-	+	-	+	-	w+	-	-	-	-	+	-	-	w+
78	3	+	+	+	+	+	-	w+	+	-	-	-	+	w+	w+	w+
79	1	-	-	-	-	w+	-	w+	-	-	-	-	-	-	-	-
79	2	-	-	-	-	+	-	w+	-	-	-	-	-	-	-	-
79	3	+	+	-	+	+	-	w+	+	-	-	-	w+	-	-	-
80	1	w+	-	-	-	+	-	w+	+	-	-	-	-	-	-	-
80	2	w+	-	-	-	+	-	w+	+	-	-	-	-	-	-	-
80	3	+	w+	-	+	+	-	w+	+	-	-	w+	-	w+	-	-
14048	1	+	+	+	-	+	-	-	+	+	+	-	+	-	-	+
14048	2	+	+	+	-	+	-	-	+	+	+	-	+	-	w+	+
14048	3	+	+	+	-	+	-	-	+	+	+	-	+	-	w+	+
14635	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14635	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14635	3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
14715	1	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+
14715	2	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
14715	3	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
15338	1	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-
15338	2	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+
15338	3	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
15468	1	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+
15468	2	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+
15468	3	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+
17749	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
17749	2	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+
17749	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
17802	1	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
17802	2	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-
17802	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
19109	1	+	-	-	-	+	+	-	+	+	-	-	-	-	-	-
19109	2	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+
19109	3	+	-	-	-	+	+	w+	+	+	+	-	-	-	-	+
19264	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
19264	2	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
19264	3	-	-	-	-	+	-	-	+	w+	-	-	-	-	-	-
23211	1	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+
23211	2	-	-	-	-	+	-	-	+	+	+	-	w+	-	-	+
23211	3	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
2588	1	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+
2588	2	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
2588	3	-	-	-	-	+	-	w+	+	+	+	-	+	-	-	+
25914	1	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
25914	2	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-
25914	3	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
25916	1	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+
25916	2	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
25916	3	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
25917	1	+	-	-	-	+	-	-	+	+	+	-	-	-	-	+
25917	2	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+
25917	3	+	-	-	-	+	+	w+	+	+	+	-	-	-	-	+
25919	1	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+
25919	2	w+	-	-	-	+	-	-	+	+	+	-	+	-	-	+
25919	3	w+	-	-	w+	+	-	w+	+	+	+	-	+	-	w+	+
25920	1	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
25920	2	+	-	-	-	+	-	-	+	+	+	-	-	-	-	+
25920	3	+	-	-	-	+	-	-	+	+	+	-	-	-	-	+
27013	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
27013	2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
27013	3	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
27043	1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
27043	2	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-
27043	3	+	-	-	-	+	-	-	-	w+	+	-	-	+	-	-

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
27562	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
27562	2	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
27562	3	+	-	-	-	+	-	-	+	w+	+	-	-	-	-	-
29988	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
29988	2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
29988	3	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
33125	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33125	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33125	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33466	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
33466	2	+	-	-	-	+	-	-	+	+	+	-	-	-	-	+
33466	3	+	-	-	-	+	-	-	+	+	+	-	+	-	-	+
33509	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
33509	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33509	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33564	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33564	2	-	+	-	-	w+	-	-	+	+	-	-	-	-	-	-
33564	3	w+	+	-	-	w+	-	-	+	+	w+	-	-	-	-	-
33653	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33653	2	-	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
33653	3	w+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
33809	1	+	+	-	-	+	-	-	+	+	-	-	-	-	-	+
33809	2	+	+	-	-	+	-	-	+	+	w+	-	-	-	-	+
33809	3	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+
33934	1	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
33934	2	+	-	-	-	+	-	-	+	w+	-	-	-	-	-	-
33934	3	+	-	-	-	+	+	-	+	+	-	-	-	-	-	-
35016	1	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+
35016	2	+	+	-	-	+	-	-	+	+	+	-	+	-	-	+
35016	3	+	+	w+	-	+	-	-	+	+	+	-	+	-	-	+
35048	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
35048	2	+	-	-	-	+	-	-	w+	-	-	-	-	-	-	-
35048	3	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-
35084	1	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
35084	2	+	-	-	-	+	-	-	+	w+	-	-	-	-	-	+
35084	3	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+
35912	1	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-
35912	2	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-
35912	3	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
43341	1	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-
43341	2	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
43341	3	+	w+	-	-	+	-	-	+	+	+	-	-	-	-	-

Appendix D continued:

Strain	Wk	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
43979	1	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+
43979	2	-	-	-	-	+	-	-	+	+	+	-	w+	-	-	+
43979	3	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
7744	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
7744	2	-	-	-	-	+	-	-	-	w+	+	-	-	-	-	w+
7744	3	-	-	-	-	+	-	-	-	w+	+	-	-	-	-	w+
9031	1	-	-	-	-	+	-	-	+	+	+	-	-	-	w+	+
9031	2	-	-	-	-	+	-	-	+	+	+	-	+	-	+	+
9031	3	-	-	-	-	+	-	-	+	+	+	-	+	-	+	+
9067	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9067	2	-	-	-	-	-	-	-	-	w+	-	-	-	-	-	-
9067	3	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
9578	1	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+
9578	2	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+
9578	3	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+

APPENDIX E

Appendix E, Table 1. Arginine Dihydrolase-Positive Study Strains included in Identification Analysis

Regional Strains	Reference Cultures
21	9031
22	15468
23	19109
24	25914
25	25916
26	33125
27	33466
28	33934
32	35048
33	43341
34	
35	
36	
54	
57	
58	
62	
63	
64	
65	

For corresponding names to ATCC and CDC numbers, see Table 2, in Materials and Methods.

Appendix E, Table 2. Arginine Dihydrolase-Negative Study Strains included in Identification Analysis

Regional Strains	Reference Cultures
1 - 20	14048
29	14635
30	15381
31	17802
37 - 53	25919
55	25920
56	27043
59	27562
60	33509
61	33564
66 - 80	33653
	33809
	35912

For corresponding names to ATCC and CDC numbers, see Table 2, in Materials and Methods.

APPENDIX F

Appendix F, Table 1. Comparisons of regional strains in cluster B of Figure 4 with the published description of *V. splendidus* biovar I

Test	no. 33125 *V. splendidus* biovar I	no. 34	no. 35
Swarming	-	-	-
Pigmentation	-	-	-
Arginine Dihydrolase	+	+	+
Oxidase	+	+	+
NO_3-NO_2	+	-	-
D-Glucose gas	-	-	-
Voges-Proskauer	-	-	-
growth at 40°C	-	-	-
Sucrose	d	-	-
Cellobiose	+	-	-
D-Gluconate	d	-	-
γ-*Amino*butyrate	-	-	-
Putrescine	-	-	-
growth at 4°C	d	+	+
growth at 20°C	+	+	+
growth at 30°C	+	-	-
growth at 35°C	d	-	-
Chitinase	+	-	+
Propionate	+	+	+
DL-Malate	+	-	-
L-Serine	+	-	-
L-Alanine	+	-	+
β-Alanine	-	-	-
D-Alanine	+	-	-
L-Leucine	-	-	-
L-Tyrosine	d	-	-
Malonate	-	-	-
L-Glutamate	+	+	+
L-Valine	-	-	-
Succinate	+	+	+
Fumarate	+	+	+

Appendix F, Table 1 continued:

Test	no. 33125 *V. splendidus* biovar I	no. 34	no. 35
L-Tartrate	-	-	-
DL-Glycerate	d	+	+
Glycine	+	-	-
DL-Aspartate	+	-	+
L-Ornithine	-	-	-
L-Citrulline	+	-	-
D-Xylose	-	-	-
L-Arabinose	-	-	-
D-Mannose	+	+	-
D-Galactose	+	+	-
D-Trehalose	+	+	-
α-Lactose	-	+	-
D-Melibiose	-	-	-
D-Glucuronate	+	-	-
Salicin	-	-	-
D-Galacturonate	-	-	-
Citrate	+	+	-
*α-Keto*glutarate	+	-	-
Sodium Pyruvate	+	+	+
Ethanol	-	-	-
Propanol	-	+	-
D-Mannitol	+	+	-
D-Sorbitol	-	-	-
meso-Inositol	-	-	-
p-Hydroxybenzoate	-	-	-
L-Histidine	d	+	-
L-Proline	+	+	-
L-Rhamnose	-	-	-
Sarcosine	-	-	-
Betaine	-	-	-
Hippurate	-	-	-
n-Acetylglucosamine	+	+	-
D-Ribose	+	+	-
D-Glucose	+	-	-
D-Fructose	+	-	-

Appendix F, Table 1 continued:

Test	no. 33125 *V. splendidus* biovar I	no. 34	no. 35
Maltose	+	+	-
Valerate	-	-	-
Heptanoate	+	-	-
DL-Lactate	+	-	+
DL-β-Hydroxybutyrate	-	-	-
Acetate	d	-	+
*iso*Valerate	-	-	-
*iso*Butyrate	-	-	-
Glycerol	d	-	+
cis-Aconitate	+	-	+
L-Threonine	+	-	+
D-Quinate	d	-	-
Benzoate	-	-	-
Hydroxymethylglutarate	N/A	-	-
Asparagine	-	-	+
Adenine	-	-	-
Xanthine	-	-	-
Caprate	d	-	-
Caprylate	-	-	-
Caproate	-	-	-
Ethanolamine	-	-	-
Glutarate	-	-	-
meso-Erythritol	-	-	-
Phenylacetate	-	-	-
Pelargonate	-	-	-

N/A = not available. d = 11-89% strains positive (Baumann *et al.* 1984).

The description of *V. splendidus* biovar I is that of Baumann *et al.* (1984), derived from four strains, except for the results for growth on asparagine, adenine, xanthine, and ethanolamine, which are from West *et al.* (1986), derived from eight strains.

An asterisk [*] beside a positive or negative result means that all strains in that cluster gave a positive or negative result, respectively, for the test. In other words, the character frequency in these cases was 100%.

Appendix F, Table 2. Comparison of regional strain 57, from subcluster C3 of Figure 4, with the published description of *V. diazotrophicus* and this study's results for *V. aestuarianus*

Test	no. 33466 *V. diazotrophicus*	no. 35048 *V. aestuarianus*	no. 57
Swarming	-	-	-
Pigmentation	-	-	-
Arginine Dihydrolase	+	+	+
Oxidase	+	+	+
NO_3-NO_2	+	+	+
D-Glucose gas	-	-	-
Voges-Proskauer	-	-	-
growth at 40°C	+	-	+
Sucrose	+	+	+
Cellobiose	+	+	+
D-Gluconate	+	+	+
γ-*Amino*butyrate	-	+	+
Putrescine	-	+	+
growth at 4°C	+	+	+
growth at 20°C	+	+	+
growth at 30°C	+	+	+
growth at 35°C	+	+	+
Chitinase	-	-	+
Propionate	-	+	+
DL-Malate	-	+	+
L-Serine	-	+	+
L-Alanine	+	+	+
β-Alanine	N/A	-	+
D-Alanine	+	+	+
L-Leucine	-	+	+
L-Tyrosine	-	+	+
Malonate	-	+	+
L-Glutamate	+	+	+
L-Valine	-	+	-
Succinate	+	+	+
Fumarate	+	+	+
L-Tartrate	N/A	+	-
DL-Glycerate	-	+	+

Appendix F, Table 2 continued:

Test	no. 33466 *V. diazotrophicus*	no. 35048 *V. aestuarianus*	no. 57
Glycine	-	+	+
DL-Aspartate	-	+	+
L-Ornithine	+	+	+
L-Citrulline	+	+	+
D-Xylose	+	+	+
L-Arabinose	+	+	+
D-Mannose	-	+	+
D-Galactose	+	+	+
D-Trehalose	+	+	+
α-Lactose	+	+	+
D-Melibiose	-	-	+
D-Glucuronate	+	+	+
Salicin	+	+	+
D-Galacturonate	+	+	-
Citrate	+	+	+
α-*Keto*glutarate	+	-	-
Sodium Pyruvate	+	+	+
Ethanol	-	+	+
Propanol	-	+	+
D-Mannitol	+	+	+
D-Sorbitol	-	+	-
meso-Inositol	-	-	+
p-Hydroxybenzoate	-	-	-
L-Histidine	+	+	+
L-Proline	+	+	+
L-Rhamnose	-	-	-
Sarcosine	-	-	+
Betaine	-	-	+
Hippurate	-	+	-
n-Acetylglucosamine	+	+	+
D-Ribose	+	+	+
D-Glucose	+	-	+
D-Fructose	+	+	+
Maltose	+	+	+
Valerate	-	-	+
Heptanoate	N/A	+	+
DL-Lactate	-	+	+
DL-β-Hydroxybutyrate	+	-	+

Appendix F, Table 2 continued:

Test	no. 33466 *V. diazotrophicus*	no. 35048 *V. aestuarianus*	no. 57
Acetate	+	-	+
*iso*Valerate	N/A	-	+
*iso*Butyrate	N/A	-	-
Glycerol	d	+	+
cis-Aconitate	N/A	-	+
L-Threonine	-	+	+
D-Quinate	N/A	-	-
Benzoate	N/A	-	-
Hydroxymethylglutarate	N/A	-	-
Asparagine	d	+	+
Adenine	-	-	-
Xanthine	-	-	-
Caprate	N/A	-	-
Caprylate	N/A	-	-
Caproate	N/A	-	-
Ethanolamine	-	-	-
Glutarate	-	-	-
meso-Erythritol	-	-	-
Phenylacetate	-	-	-
Pelargonate	-	-	+

N/A = not available. d = 16-84% strains positive.

The description of *V. diazotrophicus* is a combination of results from Guerinot *et al.* (1982), West *et al.* (1986), and Bryant *et al.* (1986). Guerinot *et al.* (1982) is the species-naming paper for *V. diazotrophicus*. It did not include all the tests run in this study, so results were obtained from the other two papers as well. The results of Guerinot *et al.* (1982) were derived from 21 strains, those of West *et al.* (1986) from two strains, and those of Bryant *et al.* (1986) from 13 strains.

The species-naming paper for *V. aestuarianus*, Tison and Seidler (1983), had results for only eight tests included in this study: swarming, arginine dihydrolase, oxidase, production of gas from the fermentation of D-glucose, the Voges-Proskauer test, and growth at 20°C, 30°C, and 35°C. The *V. aestuarianus* results in this study agreed with Tison and Seidler (1983), so these results, from Appendix C, Tables 1 to 4, were used for the comparison with regional strain 57.

Appendix F, Table 3. Comparisons of regional strains in clusters A and C of Figure 5 with descriptions of *V. cyclosites*, *V. marinus*, and *V. ordalii*

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	Cluster A	Cluster C
Swarming	-	-	-	-*	-*
Pigmentation	-	-	-	-*	-*
Arginine Dihydrolase	-	-	-	-*	-*
Oxidase	+	+	+	+*	+*
NO_3-NO_2	+	+	-	+*	d
D-Glucose gas	-	-	-	-*	-*
Voges-Proskauer	-	-	-	-*	-*
growth at 40°C	-	-	-	d	d
Sucrose	-	-	+	d	d
Cellobiose	-	-	-	d	-
D-Gluconate	-	+	-	-	-
γ-*Amino*butyrate	-	-	-	-*	-
Putrescine	-	-	-	d	-*
growth at 4°C	-	+	-	+*	+*
growth at 20°C	+	+	+	+*	+*
growth at 30°C	+	-	-	+*	+*
growth at 35°C	+	-	-	d	d
Chitinase	-	+	d	d	-*
Propionate	-	-	-	+*	+*
DL-Malate	-	+	-	-*	-*
L-Serine	-	+	-	+	d
L-Alanine	-	-	-	d	d
β-Alanine	-	-	-	-*	-
D-Alanine	-	+	-	-*	d
L-Leucine	-	-	-	d	d
L-Tyrosine	-	-	-	d	d
Malonate	-	-	-	-*	-*
L-Glutamate	-	+	+	+*	+*
L-Valine	-	-	-	-*	-
Succinate	-	+	-	+*	+*
Fumarate	-	+	-	+*	+*
L-Tartrate	-	-	-	-*	-

Appendix F, Table 3 continued:

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	Cluster A	Cluster C
DL-Glycerate	-	-	d	d	+
Glycine	-	-	-	-*	-
DL-Aspartate	-	+	+	d	d
L-Ornithine	-	-	-	-*	-
L-Citrulline	-	-	-	-*	-
D-Xylose	-	-	-	-	d
L-Arabinose	-	-	-	-*	d
D-Mannose	-	-	-	-*	-*
D-Galactose	-	+	-	-	d
D-Trehalose	-	-	-	-*	-*
α-Lactose	-	-	-	-*	-*
D-Melibiose	-	-	-	-*	-*
D-Glucuronate	-	-	-	-*	-*
Salicin	-	-	-	-*	-*
D-Galacturonate	-	-	-	-*	-*
Citrate	-	-	+	-*	d
α-*Keto*glutarate	-	+	-	-*	-*
Sodium Pyruvate	-	-	+	+*	+
Ethanol	-	-	-	-*	-
Propanol	-	-	-	-*	-*
D-Mannitol	-	-	-	d	d
D-Sorbitol	-	-	-	-*	-*
meso-Inositol	-	-	-	-*	-
p-Hydroxybenzoate	-	-	-	-*	-*
L-Histidine	-	-	d	-*	d
L-Proline	-	+	+	-*	d
L-Rhamnose	-	-	-	-*	-*
Sarcosine	-	-	-	-*	-*
Betaine	w+	-	-	-*	-*
Hippurate	-	-	-	-*	-
n-Acetylglucosamine	-	+	+	+*	d
D-Ribose	-	+	+	d	-*
D-Glucose	-	+	+	+*	+*
D-Fructose	-	+	+	+*	-
Maltose	-	+	+	+*	+*
Valerate	-	-	-	d	+*

Appendix F, Table 3 continued:

Test	no. 14635 *V. cyclosites*	no. 15381 *V. marinus*	no. 33509 *V. ordalii*	Cluster A	Cluster C
Heptanoate	-	-	-	d	d
DL-Lactate	-	+	-	+*	+*
DL-β-Hydroxybutyrate	-	-	-	-*	d
Acetate	-	+	+	d	+
*iso*Valerate	-	-	-	-*	+*
*iso*Butyrate	-	-	-	-*	-*
Glycerol	-	+	+	-*	d
cis-Aconitate	-	-	d	-*	d
L-Threonine	-	+	+	d	d
D-Quinate	+	-	-	-*	d
Benzoate	-	-	-	-*	d
Hydroxymethylglutarate	-	-	N/A	-*	d
Asparagine	-	+	N/A	d	d
Adenine	-	-	N/A	-*	-*
Xanthine	-	-	N/A	-*	-*
Caprate	-	+	-	+	+
Caprylate	-	-	-	-*	-
Caproate	-	-	-	-*	-*
Ethanolamine	-	-	N/A	-*	-*
Glutarate	-	-	-	-*	d
meso-Erythritol	-	-	-	-*	-*
Phenylacetate	-	-	-	-*	-*
Pelargonate	-	-	-	-*	-

N/A = not available. d for *V. ordalii* = 11-89% strains positive (Baumann *et al.* 1984). d for clusters A and C = 16-84% strains positive.

This study's results for *V. cyclosites* were used as the description of the species. The description of *V. marinus* is that of Baumann *et al.* (1984), derived from one strain. The description of *V. ordalii* is that of Baumann *et al.* (1984), given for *V. anguillarum* biovar II, derived from five strains.

An asterisk [*] beside a positive or negative result means that all strains in that cluster gave a positive or negative result, respectively, for the test. In other words, the character frequency in these cases was 100%.

APPENDIX G

Appendix G, Table 1. Percentage frequencies of positive characters found in individual clusters of Figure 4
Total no. of strains [regional &/or reference] given in parentheses

Test	Clusters of Figure 4 A [17]	B [3]	C [10]
Swarming	0	0	0
Pigmentation	0	0	0
Arginine Dihydrolase	100	100	100
Oxidase	100	100	100
NO_3-NO_2	100	0	90.0
D-Glucose gas	0	0	0
Voges-Proskauer	0	0	0
growth at 40°C	0	0	50.0
Sucrose	5.9	0	80.0
Cellobiose	35.3	33.3	80.0
D-Gluconate	17.6	0	80.0
γ-*Amino*butyrate	0	0	50.0
Putrescine	0	0	90.0
growth at 4°C	100	66.7	40.0
growth at 20°C	94.1	100	100
growth at 30°C	64.7	33.3	100
growth at 35°C	5.9	33.3	90.0
Chitinase	76.5	33.3	40.0
Propionate	76.5	100	100
DL-Malate	0	0	90.0
L-Serine	100	0	80.0
L-Alanine	88.2	66.7	100
β-Alanine	0	0	30.0
D-Alanine	88.2	33.3	100

Appendix G, Table 1 continued:

Test	A	B	C
L-Leucine	5.9	33.3	60.0
L-Tyrosine	41.2	33.3	100
Malonate	0	0	30.0
L-Glutamate	100	100	90.0
L-Valine	0	33.3	60.0
Succinate	100	100	100
Fumarate	100	100	100
L-Tartrate	0	0	10.0
DL-Glycerate	41.2	100	80.0
Glycine	82.4	0	70.0
DL-Aspartate	100	66.7	90.0
L-Ornithine	23.5	33.3	90.0
L-Citrulline	70.6	33.3	50.0
D-Xylose	0	0	40.0
L-Arabinose	0	33.3	60.0
D-Mannose	88.2	33.3	50.0
D-Galactose	100	33.3	40.0
D-Trehalose	88.2	33.3	100
α-Lactose	100	33.3	40.0
D-Melibiose	17.6	0	20.0
D-Glucuronate	29.4	0	40.0
Salicin	0	0	60.0
D-Galacturonate	0	0	30.0
Citrate	100	66.7	90.0
α-*Keto*glutarate	0	0	0
Sodium pyruvate	100	100	100
Ethanol	5.9	33.3	60.0
Propanol	5.9	66.7	60.0

Appendix G, Table 1 continued:

Test	A	B	C
D-Mannitol	100	33.3	90.0
D-Sorbitol	0	0	50.0
meso-Inositol	0	0	20.0
p-Hydroxybenzoate	0	0	0
L-Histidine	94.1	66.7	80.0
L-Proline	100	66.7	100
L-Rhamnose	0	0	10.0
Sarcosine	0	0	10.0
Betaine	0	0	20.0
Hippurate	0	0	20.0
n-Acetylglucosamine	100	66.7	100
D-Ribose	88.2	33.3	90.0
D-Glucose	17.6	33.3	70.0
D-Fructose	23.5	0	90.0
Maltose	88.2	33.3	100
Valerate	11.8	0	50.0
Heptanoate	0	0	90.0
DL-Lactate	100	33.3	100
DL-β-Hydroxybutyrate	0	0	60.0
Acetate	76.5	33.3	90.0
*iso*Valerate	0	0	60.0
*iso*Butyrate	0	0	40.0
Glycerol	100	33.3	90.0
cis-Aconitate	100	33.3	20.0
L-Threonine	100	33.3	70.0
D-Quinate	0	0	0
Benzoate	0	0	0
Hydroxymethylglutarate	0	0	0

Appendix G, Table 1 continued:

Test	A	B	C
Asparagine	100	33.3	100
Adenine	5.9	0	20.0
Xanthine	0	0	0
Caprate	64.7	0	100
Caprylate	0	0	80.0
Caproate	0	0	70.0
Ethanolamine	0	0	0
Glutarate	0	0	30.0
meso-Erythritol	0	0	0
Phenylacetate	11.8	0	10.0
Pelargonate	0	0	60.0

Appendix G, Table 2. Percentage frequencies of positive characters found in individual clusters of Figure 5.
Total no. of strains [regional &/or reference] given in parentheses

Test	Clusters of Figure 5							
	A [12]	B [6]	C [7]	D [14]	E [9]	F [7]	G [8]	H [10]
Swarming	0	0	0	0	0	0	0	0
Pigmentation	0	0	0	0	0	0	0	0
Arginine Dihydrolase	0	0	0	0	0	0	0	0
Oxidase	100	100	100	92.9	100	85.7	100	100
NO_3-NO_2	100	50.0	71.4	0	100	28.6	50.0	100
D-Glucose gas	0	0	0	0	0	0	0	0
Voges-Proskauer	0	0	0	0	0	0	0	0
growth at 40°C	16.7	16.7	28.6	0	22.2	28.6	87.5	70.0
Sucrose	75.0	16.7	42.9	85.7	0	57.1	62.5	40.0
Cellobiose	58.3	16.7	14.3	85.7	33.3	14.3	75.0	60.0
D-Gluconate	8.3	33.3	14.3	78.6	22.2	28.6	100	80.0
γ-*Amino*butyrate	0	16.7	14.3	14.3	0	0	100	40.0
Putrescine	33.3	0	0	0	0	0	25.0	30.0
growth at 4°C	100	66.7	100	100	88.9	100	100	0
growth at 20°C	100	100	100	100	100	100	100	100
growth at 30°C	100	66.7	100	100	66.7	100	100	100
growth at 35°C	16.7	16.7	42.9	21.4	44.4	57.1	87.5	100
Chitinase	41.7	16.7	0	0	22.2	0	0	50.0
Propionate	100	50.0	100	100	100	100	100	100
DL-Malate	0	33.3	0	14.3	0	28.6	62.5	80.0
L-Serine	91.7	33.3	71.4	100	100	100	75.0	100
L-Alanine	75.0	33.3	71.4	100	100	100	100	90.0
β-Alanine	0	0	14.3	7.1	0	14.3	87.5	30.0
D-Alanine	0	66.7	42.9	28.6	100	57.1	100	90.0

Appendix G, Table 2 continued:

Test	A	B	C	D	E	F	G	H
L-Leucine	33.3	33.3	57.1	92.9	44.4	100	100	30.0
L-Tyrosine	16.7	50.0	42.9	100	55.6	85.7	100	70.0
Malonate	0	0	0	7.1	0	28.6	100	20.0
L-Glutamate	100	83.3	100	100	100	100	100	100
L-Valine	0	16.7	14.3	7.1	0	28.6	75.0	50.0
Succinate	100	83.3	100	100	100	100	100	100
Fumarate	100	66.7	100	100	100	100	100	100
L-Tartrate	0	0	14.3	0	0	14.3	0	10.0
DL-Glycerate	83.3	16.7	85.7	14.3	66.7	57.1	87.5	70.0
Glycine	0	16.7	14.3	85.7	100	42.9	87.5	70.0
DL-Aspartate	16.7	66.7	42.9	92.9	100	85.7	62.5	100
L-Ornithine	0	16.7	14.3	50.0	22.2	0	62.5	90.0
L-Citrulline	0	16.7	0	100	55.6	100	37.5	60.0
D-Xylose	8.3	0	28.6	92.9	11.1	71.4	25.0	20.0
L-Arabinose	0	0	57.1	7.1	0	42.9	37.5	70.0
D-Mannose	0	33.3	0	100	100	100	75.0	80.0
D-Galactose	8.3	50.0	28.6	100	100	100	62.5	60.0
D-Trehalose	0	16.7	0	50.0	100	100	62.5	100
α-Lactose	0	0	0	100	100	85.7	62.5	10.0
D-Melibiose	0	0	0	100	11.1	100	50.0	20.0
D-Glucuronate	0	0	0	7.1	66.7	14.3	62.5	30.0
Salicin	0	0	0	0	0	100	12.5	40.0
D-Galacturonate	0	0	0	0	0	0	12.5	20.0
Citrate	0	16.7	28.6	92.9	100	100	100	80.0
α-*Keto*glutarate	0	16.7	0	0	0	0	0	0
Sodium pyruvate	100	50.0	85.7	100	100	100	100	100
Ethanol	0	16.7	14.3	0	0	85.7	100	60.0
Propanol	0	16.7	0	7.1	0	85.7	100	60.0

Appendix G, Table 2 continued:

Test	A	B	C	D	E	F	G	H
D-Mannitol	83.3	33.3	28.6	100	100	100	100	90.0
D-Sorbitol	0	0	0	0	0	85.7	12.5	40.0
meso-Inositol	0	0	14.3	0	0	100	62.5	20.0
p-Hydroxybenzoate	0	0	0	21.4	0	100	12.5	20.0
L-Histidine	0	33.3	28.6	0	100	100	62.5	70.0
L-Proline	0	50.0	28.6	85.7	100	100	100	100
L-Rhamnose	0	0	0	0	0	100	25.0	10.0
Sarcosine	0	0	0	0	0	85.7	50.0	10.0
Betaine	0	0	0	0	0	100	87.5	20.0
Hippurate	0	0	14.3	0	0	42.9	0	10.0
n-Acetylglucosamine	100	33.3	71.4	14.3	100	100	62.5	100
D-Ribose	16.7	83.3	0	14.3	77.8	85.7	87.5	90.0
D-Glucose	100	33.3	100	100	0	100	100	60.0
D-Fructose	100	66.7	14.3	100	0	100	100	70.0
Maltose	100	33.3	100	100	88.9	100	100	90.0
Valerate	41.7	16.7	100	85.7	0	42.9	100	30.0
Heptanoate	16.7	0	42.9	92.9	0	42.9	100	90.0
DL-Lactate	100	33.3	100	7.1	100	42.9	100	100
DL-β-Hydroxybutyrate	0	0	71.4	7.1	0	14.3	100	50.0
Acetate	25.0	16.7	85.7	100	100	100	100	80.0
*iso*Valerate	0	16.7	100	0	0	14.3	100	20.0
*iso*Butyrate	0	0	0	0	0	14.3	12.5	30.0
Glycerol	0	33.3	42.9	21.4	100	42.9	87.5	100
cis-Aconitate	0	16.7	71.4	78.6	100	85.7	100	10.0
L-Threonine	41.7	33.3	57.1	100	100	100	50.0	70.0
D-Quinate	0	16.7	28.6	7.1	0	14.3	0	30.0
Benzoate	0	0	42.9	7.1	0	14.3	12.5	10.0
Hydroxymethylglutarate	0	0	42.9	7.1	0	14.3	0	0

Appendix G, Table 2 continued:

Test	A	B	C	D	E	F	G	H
Asparagine	16.7	33.3	57.1	78.6	100	57.1	100	90.0
Adenine	0	0	0	0	11.1	0	0	0
Xanthine	0	0	0	0	0	14.3	0	0
Caprate	91.7	16.7	85.7	78.6	77.8	100	100	90.0
Caprylate	0	0	14.3	21.4	0	0	25.0	70.0
Caproate	0	0	0	0	0	0	0	70.0
Ethanolamine	0	0	0	0	0	14.3	12.5	0
Glutarate	0	16.7	42.9	0	0	28.6	50.0	20.0
meso-Erythritol	0	0	0	0	0	14.3	0	10.0
Phenylacetate	0	0	0	0	0	28.6	0	0
Pelargonate	0	0	14.3	71.4	11.1	71.4	100	50.0