A Numerical Taxonomic and Serological Study of *Listeria* and Possibly Related Bacteria.

by

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STATEMENT.

The work described in this thesis was carried out by the author in the Medical Research Council, Microbial Systematics Unit at the University of Leicester in the period October 1970 to September 1973. The work has not been presented and is not being currently presented for any other degree.

Signed:

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The Medical Research Council,
Microbial Systematics Unit,
The University,
Leicester.

To my husband for his patience
and understanding, and my parents
for their unfailing encouragement.

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SUMMARY.

A numerical taxonomic survey has been performed using 192 strains of *Listeria* and possibly related bacteria. Of these, 38 strains have been further investigated by serological techniques.

The results indicate that *Listeria monocytogenes* is a good, homogeneous species. *L. grayi* and *L. murrayi* do not differ sufficiently from each other to warrant individual specific status, and it is recommended that they be combined in the single species *L. grayi*. They show a close relationship to *L. monocytogenes*, compatible with that of two species within the same genus, but *L. denitrificans* differs greatly from the other *Listeria* spp. and should be removed from the genus.

Erysipelothrix, *Listeria* and *Microbacterium thermosphactum* show a closer resemblance to the family *Lactobacillaceae* than to the family *Corynebacteriaceae* where they are at present classified. The suggested relationship of *Gemella haemolysans* to the tribe *Streptococceae* in the *Lactobacillaceae* has been confirmed, and it is possible to envisage an enlarged family *Lactobacillaceae* including *Listeria*, *Erysipelothrix*, *M. thermosphactum*, *G. haemolysans* and possibly the bacteria refered to as *Bacterium eurydice*. Alternatively, *Erysipelothrix*, *M. thermosphactum*, *G. haemolysans* and possibly *B. eurydice* could be placed in the *Lactobacillaceae*, and a new family, possibly the *Listeriaceae*, could be introduced for *Listeria* and the many lactic acid bacteria which appear to be related to *Listeria*, but have not been assigned to an exact taxonomic position.

CONTENTS.

INTRODUCTION.

INTRODUCTION.

Listeria is one of a number of Gram positive, non-sporing. non-acid-fast, non-mycelial rods which may be catalase positive or negative, of which the exact taxonomic position is not clear. Bacteria of this description are usually allocated to one of four families included in the seventh edition of Bergey's Manual of Determinative Bacteriology (1957), the Lactobacillaceae, Corynebacteriaceae, Brevibacteriaceae or Propionibacteriaceae.

The main criterion determining allocation to the family Lactobacillaceae has been lack of demonstrable catalase, although this has been shown to be inconsistent (Dacre and Sharpe (1956), Vaňková (1957) and Whittenbury (1964).

The family Corynebacteriaceae is the repository for Gram positive, catalase positive rods which show a characteristic pleomorphism known as "coryneform" or "diphtheroid" morphology. Here again there are anomalies, in that at least two genera, Listeria and Erysipelothrix, do not exhibit this characteristic pleomorphism, and Erysipelothrix, in fact, is even more peculiarly placed as it is catalase negative! One can speculate that the placing of this latter genus in the family Corynebacteriaceae was coloured by the fact that it is pathogenic. The medical microbiologists would be more tempted to place it with other disease-causing rods, than with the genus Lactobacillus.

The predetermining character of the Propionibacteriaceae is the production of propionic acid.

The fourth family, Brevibacteriaceae, contains two genera,

Kurthia and Brevibacterium, and tends to be even more of a miscellaneous collection than the other three families. The genus Kurthia is reasonably well defined (Gardner (1969)), but the genus Brevibacterium is used as a home for any small Gram positive rod which cannot be placed elsewhere.

The genus Listeria is presently classified in the family Corynebacteriaceae, but many microbiologists (Murray (1962), Jones, Sneath and Woodbine (1966)) think that it could equally well be placed in the family Lactobacillaceae, and with some reservations in either of the other two families.

Listeria is a clinically important genus; one species, L. monocytogenes, is known to be the causal agent of a variety of clinical symptoms (Seeliger (1961), Gray and Killinger (1966) and Bojsen-Møller (1972)). For many years listeriosis does not seem to have been reported with the same frequency in the U.K. as it has been in the U.S.A. and Europe. From the time of its characterisation by Murray, Webb and Swann (1926), workers have commented upon the difficulty of isolation of Listeria, and perhaps the low incidence in the U.K. is more apparent than real (Mair (1968), Kramer and Jones (1969)).

L. monocytogenes is known to grow well at 4°C and under conditions of reduced oxygen tension. With the increasing use of prepackaging, and low-temperature holding of meat products, Microbacterium thermosphactum has been isolated with rising frequency. The same conditions could favour the growth of L. monocytogenes (Kramer and Jones (1969)). Additionally, a number of Gram positive bacteria, which have been described as atypical lactobacilli, have been isolated from chicken meat by Thornley and Sharpe (1959) and Barnes (pers.

comm.). With the increasing use of prepackaging, and in view of the potential risk of Listeria developing in prepacked food held at low temperature, it seemed important to investigate the taxonomic position of the genus Listeria, with particular attention to its relationship to other Gram positive, lactic acid producing bacteria. For this investigation it was important to consider members of the families Corynebacteriaceae, Lactobacillaceae, Propionibacteriaceae and Brevibacteriaceae, and also the family Micrococcaceae which contains a group of Gram positive fermentative cocci.

Corynebacteriaceae.

The family Corynebacteriaceae was first proposed by Lehmann and Neumann in 1907, to contain the genus Corynebacterium which they had named in 1896. As described in the seventh edition of Bergey's Manual (1957), the bacteria are Gram positive, catalase positive, non-sporing, non-acid-fast rods, which are irregular in size and exhibit "coryneform" or "diphtheroid" pleomorphism, characterised by the formation of club-shaped and wedge-shaped cells. They may be readily decolourized, and may only show Gram positive inclusions, or granules, in otherwise Gram negative cells. Oxygen requirements range from aerobic to microaerophilic and a few species are anaerobic. The family includes animal and plant parasites and pathogens, as well as isolates from dairy products and the soil.

There are at present six genera in the family Corynebacteriaceae, they are Arthrobacter, Cellulomonas, Corynebacterium, Listeria, Erysipelothrix and Microbacterium.

Arthrobacter.

The genus Arthrobacter is composed of bacteria which show little or no fermentation of carbohydrates, and is, therefore of little importance in a study centred around the lactic acid bacteria.

Cellulomonas.

This genus is distinguished from the other soil genus, Arthrobacter, by its ability to attack cellulose, and to ferment carbohydrates. Cellulomonas was, therefore, a stronger candidate for study than the genus Arthrobacter, particularly in view of a report by Chatelain and Second (1966) that Listeria denitrificans is cellulolytic.

Corynebacterium.

When first proposed by Lehmann and Neumann for the diphtheria bacillus, this was a pleasingly simple genus, containing only animal pathogenic strains. Both plant pathogenic and saprophytic bacteria have since been added, on the basis of their morphology, and the situation is far from simple. As early as 1947, Conn and Dimmick complained of the misuse of the term Corynebacterium (Conn and Dimmick (1947)). Since then many workers have called for a reappraisal of the position of the genus Corynebacterium, for example, da Silva and Holt (1965), Davis and Newton (1969), Bousfield (1972) and Vanderzant et al. (1972). Jensen (1952), pointed out that, "There are perhaps few groups of bacteria of which the typical representatives are easier to recognize and the aberrant types more numerous and more difficult to separate than those which originally constituted the genus Corynebacterium, and which we now may cautiously call the group of

coryneform bacteria".

It has been suggested (Seeliger (1961)), that some of the plant pathogenic species of Corynebacterium bear a close resemblance to Listeria; and C. poinsettiae, although not closely related to Listeria, can often be mistaken for this species (Seeliger (1961)).

Listeria.

Listeria was first accurately described by Murray, Webb and Swann (1926) after an epizootic among the laboratory breeding stock of rabbits at Cambridge. A pure culture was isolated and named Bacterium monocytogenes in recognition of the monocytosis observed in the rabbits.

There is little doubt that this bacterium had already been described several times, especially from clinical cases; for example, Hayem in France as early as 1891 and Henle in Germany in 1893, observed Gram positive rods in tissue sections of patients who had almost certainly died from listeric infections (Gray and Killinger (1966)). Hulpers (1911), in Sweden, isolated and named Bacterium hepatis, which in many ways resembles Listeria. Some workers prefer to attribute the discovery of Listeria to Hulpers, but this has been opposed because there are several discrepancies between the descriptions of the biochemical properties of B. hepatis and L. monocytogenes. Since the original isolates of B. hepatis have long been lost, this dispute will never be settled. A culture isolated by Dumont and Cotoni (1921), which was maintained at the Pasteur Institute in Paris was identified, at a much later date, as L. monocytogenes.

A year after Murray et al. (1926) had published their

findings, Pirie (1927) described what was soon found to be the same bacterium. He named it Listerella hepatolytica in honour of Lord Lister, and in recognition of the necrotic foci found in the livers of the gerbils (Iatera lobenquiae) from which it was isolated.

The unsuitability of the generic name Bacterium, led to the proposal by Pirie, and the subsequent acceptance, of the composite name Listerella monocytogenes. In 1939 the Committee on Nomenclature, at the Third International Congress for Microbiology, New York, resolved that in all duplications of generic names only the first one applied should be considered valid. The generic name Listerella had already been applied to both a mycetozoan, and a species of foraminifera, and therefore could not be used to describe a bacterium. Pirie (1940), therefore, suggested the name Listeria, which was adopted in the sixth edition of Bergey's Manual (1948), and approved by the Judicial Commission on Bacteriological Nomenclature and Taxonomy (Judicial Commission (1954)).

Nyfeld (1929) reported the first confirmed case of a listeric infection of man, initially believing it to be a new variant, he named it Listeria monocytogenes var hominis, but it was not in fact culturally or biochemically unique and the name was dropped. Similarly, such names as Listeria ovis, L. gerbilli, L. cuniculi etc., based upon the source of isolation, have been proved to have no taxonomic standing.

For many years, therefore, Listeria was in the anomalous position of being a monospecific genus. It was defined in the seventh edition of Bergey's Manual (1957) as a genus of Gram positive bacteria which are motile by means of

peritrichous flagellae, and which can vary morphologically from coccobacilli, through all lengths of rods, to filaments which may be up to 200 μ long. They are aerobic to facultatively anaerobic, catalase positive, and able to hydrolyse aesculin and to produce acid, but not gas, from glucose and a few additional carbohydrates. Pathogenisis can occur causing a variety of symptoms; in man, infectious mononucleosis, meningitis and encephalitis; and human neonates can be infected *in utero* as a result of inapparent infection in the mother. The host range is wide, including at least 37 mammals other than man, 17 fowls, ticks, fish and crustaceans (Gray (1963)). In addition, *Listeria* has a world-wide distribution in such diverse habitats as the soil, stream water, slaughter house waste, sewage and silage, as well as both human and animal carriers, apparently unaffected by infection.

More recently further species have been suggested, Larsen and Seeliger (1966) proposed that a strain from chinchilla faeces should be assigned to a new species, mainly on the basis of its fermentation of mannitol, and the name *L. grayi* was proposed in honour of the work performed by M.L.Gray. At the same time they proposed that the non-pathogenic, non-haemolytic strain which fermented arabinose, but not mannitol, and reduced nitrate, L26, isolated by Sohier et al., (1948), should be given species status, proposing the name *L. denitrificans*. The fourth, and as yet final species, *L. murrayi*, is non-pathogenic, non-haemolytic, ferments mannitol and reduces nitrate. It was isolated from vegitation by Welshimer and Meredith (1971), and was named in honour

of the late E.G.D.Murray, co-discoverer of L. monocytogenes. TABLE 1 (based upon information from Seeliger (1961), Welshimer and Meredith (1971) and Jones (pers. comm.)) is an attempt to summarize the main characters of the four Listeria species.

L. denitrificans differs in other ways from the other three species, it is morphologically distinct, in that Gram stains often show deeply staining coccoid inclusions, but free coccoidal bodies have never been observed. Chatelain and Second (1966) reported that L. denitrificans is cellulolytic, and its DNA base composition of 56 moles % GC (Welshimer and Meredith (1971)) is obviously distinct from the value of 38 moles % GC obtained for the other three species (see TABLE 2).

There are four main serotypes of L. monocytogenes, first described by Paterson (1940), they are based upon both somatic 'O' and flagella 'H' antigens and there are further subdivisions within the four types (Seeliger (1961), Gray and Killinger (1966)). Welshimer and Meredith (1971) investigated the serological relationships within the genus Listeria. L. denitrificans failed to agglutinate 'O' antisera against L.monocytogenes, L. grayi or L. murrayi, indicating a complete lack of common 'O' antigens. They also found that L. murrayi lacked common 'H' antigens with L. monocytogenes, and that with L. murrayi 'O' antiserum L. monocytogenes serotypes 3 and 4a reacted at low titres, and serotypes 1 and 4b were non-reactive. L. murrayi and L. grayi, however, possessed common 'O' and 'H' antigens; a minimum of three 'H' antigens were found and a complex mixture of 'O' antigens, shared and

TABLE 1. A comparison of some important characteristics of the four species of Listeria. (Based upon Seeliger (1961), Welshimer and Meredith (1971) and Jones (pers. comm.)).

Characters	L. monocytogenes	L. grayi	L. murrayi	L. denitrificans
Anton eye test	+	+	-	-
Catalase	+	+	+	+
Motility	+	+	+	+
β Haemolysis	+	+	-	-
Voges-Proskauer test	+	+	+	-
Methyl Red test	+	+	+	+
Nitrate reduction	-	-	+	+
Aesculin hydrolysis	+	+	+	+
Acid production from-				
adonitol	-	-	-	+
L-arabinose	-	-	-	+
cellobiose	+	+	+	+
dextrin	+	+	+	+
dulcitol	-	-	-	-
erythritol	-	-	-	-
D-galactose	V/D	+	+	+
glucose	+	+	+	+
glycogen	-	-	-	-
inositol	-	-	-	-
inulin	-	-	-	-
lactose	V/D	+	+	+

TABLE 1. (continued).

Characters	L. monocytogenes	L. grayi	L. murrayi	L. denitrificans
Acid production from-				
laevulose	+	+	+	+
maltose	+	+	+	+
mannitol	-	+	+	-
mannose	+	+	+	+
melibiose	-	-	-	+D
melizitose	V	-	-	-
raffinose	-	-	-	-
rhamnose	+*	-	+o	-
salicin	+	+	+	+
starch	+	+	+	+
sucrose	V/D	-	-	+
trehalose	+	+	+	+D
xylose	-	-	-	-

\+ acidification in 48 hours

V variable

D delayed acidification

* rare strains of L. monocytogenes are rhamnose negative.

o six out of nine strains were positive

TABLE 2. The DNA Base Compositions of the four species of Listeria. (Determined from Thermal Melting Point (T_m) and Buoyant Density values by Stuart and Welshimer (1973) and expressed as moles % guanine + cytosine / moles of total bases (moles % GC).

Species	T_m	Buoyant density
Listeria monocytogenes	37 - 38	38 - 39
Listeria grayi	39	41
Listeria murrayi	38 - 39	42
Listeria denitrificans	56	57

unshared by L. murrayi and L. grayi, were indicated.

Stuart and Welshimer (1973), studied the DNA : DNA hybridization patterns within the genus Listeria. The results indicated two groups, independant of source or serotype, within the species L. monocytogenes, one which showed 80 to 100% binding to the DNA of the type strain, the other 40 to 70% binding. L. monocytogenes and L. denitrificans were clearly distinct from each other and the other two species. The DNA of L. murrayi and L. grayi, however, exhibited 58 to 76% binding to each other, compared with 71 to 100% binding within the two separate species.

Erysipelothrix.

The first member of this genus, Erysipelothrix muriseptica, was found by Koch (1880) in the blood of mice which had been injected subcutaneously with putrifying blood. Pasteur à Dumas (1882) briefly described a bacterium isolated from pigs dying of rouget (swine erysipelas), and Pasteur and Thuiller (1883) used this strain when they first demonstrated the method of artificial immunization using live attenuated bacteria. It was Loeffler (1886), however, who first accurately described the causal agent of swine erysipelas. Rosenbach (1909) suggested the names E. porci, E. muriseptica and E. erysipeloides for pig, mouse and human isolates respectively. Rickman (1909), however, proposed that the cultural and morphological differences between the three species were insufficient, and since they all reacted equally to immune sera he concluded that they were no more than variants of a single species (Woodbine (1950)).

The bacterium was named Bacillus insidiosus by Trevisan

(1885) and E. rhusiopathiae by Winslow et al. (1920). The latter name became firmly established in the literature, and the former was not used again until Langford and Hansen (1953) challenged the use of the name E. rhusiopathiae, and proposed Erysipelothrix insidiosa (Trevisan) comb. nov. The Langford and Hansen name was not generally accepted until the seventh edition of Bergey's Manual (1957), when Langford and Hansen were responsible for the description of the genus Erysipelothrix. They recognised one species only, E. insidiosa (Trevisan) Langford and Hansen (1953); but the name (insidiosa) had not appeared in the literature for at least 63 years! Shuman and Wellmann(1966) discussed the status of the nomenclature of Erysipelothrix, and requested an opinion from the Judicial Commission on Bacterial Nomenclature to conserve the specific epithet rhusiopathiae. In 1967 the Judicial Commission considered the request and there were no objections (Judicial Commission (1967)). The conservation of rhusiopathiae in the name E. rhusiopathiae (Migula (1900)) Buchanan (1918), against insidiosa (basionym: Bacillus insidiosa Trevisan (1885)) was passed in 1970 (Judicial Commission (1970)).

Erysipelothrix is described, in the seventh edition of Bergey's Manual (1957), as a group of Gram positive non-sporing, non-acid-fast bacteria, which vary from coccobacilli to filamentous forms. The filaments are narrower than those of Listeria, and the bacteria are non-motile, facultatively anaerobic, catalase negative and fermentative. Fewer sugars are attacked than by Listeria, but like Listeria no gas is produced.

Surprisingly, the apparent lack of cytochromes and catalase was not considered sufficient reason for excluding the genus from the family Corynebacteriaceae, although the presence of catalase and cytochrome was fundamental in the exclusion of Microbacterium thermosphactum from the family Lactobacillaceae!

There have been many proponents of a close relationship between Listeria and Erysipelothrix, basically because of their morphological and pathological similarities. Barber (1939), after comparing several strains of Erysipelothrix and Listeria monocytogenes concluded that Erysipelothrix behaved like a Listeria with reduced synthetic powers, exhibited a similar morphology, was also pathogenic, but showed no antigenic similarities. Hutner (1942) felt that his results supported Barber's theory; in studying the growth requirements of the two bacteria he found that minimal media for them were very similar, Listeria merely showed fewer requirements. Perhaps the most notable exponents of a close relationship between these bacteria are Miles and Wilson (Topley and Wilson (1964), who included both genera within the same section. They proposed the adoption of the name Erysipelothrix monocytogenes, but continued to use the generic name Listeria out of deference to their fellow bacteriologists. They thought that the differences between Listeria and Erysipelothrix were only of the inter specific type, and that it was a violation of the principles of bacterial taxonomy to place them in different genera. Some characters of Listeria and Erysipelothrix are compared in TABLE 3, based upon Julianelle (1941), Sohier (1948), Füzi (1963), Topley and Wilson (1964) and Grieco and

TABLE 3. Characters which distinguish Erysipelothrix rhusiopathiae from Listeria monocytogenes. (Based upon Julianelle (1941)[1], Sohier (1948)[2], Füzi (1963)[3], Topley and Wilson (1964)[4] and Grieco and Sheldon (1970)[5]).

Characters		Erysipelothrix rhusiopathiae	Listeria monocytogenes
Size of rods	4	slender	thicker
Motility	4	-	+
Growth in gelatin stab	4	'lampbrush'	'filiform'
Haemolysis	4	α	β
Soluble haemolysin	4	-	+
Haemagglutination	4	+ *	-
Growth at 4°C	4	-	+
Neomycin sensitivity	3	-	+
Potassium tellurite sensitivity	5	+	-
Aesculin hydrolysis	2,4	-	+
Catalase activity	4	-	+
Methyl Red test	4	-	+
Voges-Proskauer test	4	-	+
Acid production from-			
galactose	1,5	+	-
maltose	1,5	-	+
mannose	1,5	-	+
rhamnose	1,5	-	+
salicin	1,5	-	+
sucrose	1,5	-	+

TABLE 3. (continued).

Characters	Erysipelothrix rhusiopathiae	Listeria monocytogenes
Acid production from-		
trehalose [5]	-	+
Antigenic cross-reaction [4]	-	-
Pathogenicity-		
kills pigeons [4]	+	-
kills guinea pigs [4]	-	+
Corneal reaction (rabbit) [1]	-	+
Site of invasion [5]	extracellular	intracellular

* human and some animal cells.

1 Julianelle (1941).

2 Sohier (1948).

3 Füzi (1963).

4 Topley and Wilson (1964).

5 Grieco and Sheldon (1970).

Sheldon (1970). Also of interest are the results of Parnas et al. (1966) who showed that strains of Erysipelothrix and Listeria possed almost identical infrared spectra.

Most authorities (Bergey's Manual (1957) and Seeliger (1961)) supported the separation of the two genera, on the basis of their antigenic dissimilarity, as demonstrated by Julianelle (1941), Seeliger (1961) and Nelson and Shelton (1963). The production of acid from some sugars (e.g., galactose) by Erysipelothrix and not by Listeria tended to contradict the ideas of Barber (1939) and Hutner (1943). Recent work by Davis, Fomin, Wilson and Newton (1969) and Stuart and Pease (1972) indicated a closer relationship between Erysipelothrix and various streptococci than between Erysipelothrix and Listeria , or the other members of the family Corynebacteriaceae. Erysipelothrix, therefore, was an obvious candidate for inclusion in the present study.

Microbacterium.

The genus Microbacterium was proposed by Orla-Jensen (1919) for a collection of small, Gram positive, non-motile, non-sporing, rod-shaped bacteria which exhibited an unusual degree of heat resistance. The taxonomic position of the genus has always been uncertain - in the fifth edition of Bergey's Manual (1939) it was placed in the family Bacteriaceae; in the sixth edition (Bergey's Manual(1948)) it was transfered to the Lactobacillaceae, and in the seventh edition (Bergey's Manual (1957)) to the family Corynebacteriaceae.

The type species, M. lacticum, was named by Orla-Jensen in 1919, together with M. flavum and M. liquifaciens; later, M. thermosphactum (McLean and Sulzbacher (1953)) and M. mobile

(Bolcato (1957)) were proposed. M. mobile was created for a group of motile lactic acid bacteria which Bolcato (1957) thought resembled the genus Lactobacillus in morphology, some secondary characters and fermentative properties, but could not be placed in that genus because of their motility and production of catalase. At the time, the genus Microbacterium was classified in the family Lactobacillaceae, and lactic acid producing, rod-shaped bacteria were split between the genera Lactobacillus and Microbacterium on the basis of their catalase activity. Very little is known about M. mobile and the original cultures appear to be unobtainable.

M. thermosphactum was recognised to be a lactic acid bacterium by McLean and Sulzbacher (1953) and they thought that, on the basis of its low heat resistance and morphology, it closely resembled members of the genus Lactobacillus, but like M. mobile it was placed in the genus Microbacterium on the basis of its catalase production.

The taxonomic position of the genus has always been uncertain and many attempts have been made to clarify the situation, notably by, Jensen (1934), Abd-el-Malek and Gibson (1952), Deibel and Evans (1960), Robinson (1966 a and b), Barlow and Kitchell (1966), Jayne-Williams and Skerman (1966) Davidson and Hartree (1968), Davidson, Mobbs and Stubbs (1968), Davis and Newton (1969), Davis, Fomin, Wilson and Newton (1969) and Collins-Thompson, Sørhaug, Witter and Ordal (1972).

As early as 1934, Jensen proposed that M. flavum was an intermediate between Mycobacterium and Corynebacterium, and that M. lacticum and M. liquifaciens should be placed in the

genus Corynebacterium. Doetsch and Rakosky (1950) believed that their results indicated that M. liquifaciens was practically identical to M. lacticum, and recommended that it should be considered as a variety of M. lacticum. Abd-el-Malek and Gibson (1952) studied thermoduric isolates from milk and concluded that many were identifiable as M. lacticum. They reported that the characteristics of these strains, and some identifiable as M. liquifaciens, were typical of the Corynebacteriaceae. As a result of their findings, and in acceptance of the proposal of Jensen (1934), Abd-el-Malek and Gibson adopted the nomenclature Corynebacterium lacticum and Corynebacterium liquifaciens.

Robinson (1966 b) proposed the removal of M. flavum to the genus Corynebacterium because of the identical nature of its cell wall composition and the similarity of enzyme patterns in starch gel to those of the human and animal pathogenic corynebacteria. He also commented that M. lacticum and M. liquifaciens appeared very similar, but that their taxonomic position was not clear. Collins-Thompson et al. (1972) also supported the removal of M. flavum to the genus Corynebacterium, on the basis of the DNA base ratios (M. flavum 58 moles % GC; Corynebacterium 48 to 59 moles % GC) and metabolism. Schleifer (1970) found that M. lacticum contained an unfamiliar cell wall murein pattern, like that of the plant pathogenic corynebacteria, possible evidence that it too should be transfered to the family Corynebacteriaceae, and consistent with the earlier suggestions of Jensen (1934) and Abd-el-Malek and Gibson (1952).

Barlow and Kitchell (1966) reported that although

M. thermosphactum resembled M. lacticum in many ways, it did not exhibit "coryneform" morphology and was not heat resistant. It did, however, share certain characters with the homofermentative lactobacilli, but was catalase positive and would not grow on Rogosa agar. They also agreed with the findings of Deibel and Evans (1960) that M. thermosphactum was negative in the benzidine test.

Davidson, Mobbs and Stubbs (1968) thought that despite the above reports, and on the basis of a positive catalase reaction, morphological characteristics and possession of functional cytochromes, M. thermosphactum should be retained in the family Corynebacteriaceae, rather than being transfered to the family Lactobacillaceae. This view was supported by Davidson and Hartree (1968) who suggested that their isolation of cytochrome and a cyanide-sensitive NADH-oxidase from M. thermosphactum supported its retention in the family Corynebacteriaceae.

Collins-Thompson et al. (1972) investigated the DNA base ratios of the genus Microbacterium, and found much variation. M. thermosphactum (36 moles% GC) resembled many of the lactic acid bacteria (e.g., Streptococcus and Listeria), M. lacticum (64 moles% GC) resembled the genus Arthrobacter, and the value for M. flavum (58 moles% GC), as previously remarked, coincided with the values quoted for the corynebacteria, heterogeneous as they are. They also found that M. thermosphactum differed from M. lacticum and M. flavum in its enzyme complement, it showed a higher activity in the catabolism of glucose to lactic acid, possed different esterase enzymes and was almost completely lacking in T.C.A. enzymes.

This latter feature is reminiscent of *Listeria* which was reported by Trivett and Meyer (1971) to have a split, non-cyclic citrate pathway.

Collins-Thompson et al. recognised the similarity of the DNA base ratio of *M. thermosphactum* to the family *Lactobacillaceae*, but proposed that the presence of catalase activity precluded its transfer to the family. In support of this proposal they cited the findings of Shaw and Stead (1970), that the lipid composition of *M. thermosphactum* was relatively similar to those of *M. lacticum* and the genus *Arthrobacter*, and was therefore, incompatible with the family *Lactobacillaceae*, but compatible with the family *Corynebacteriaceae*. They also quoted the work of Schleifer (1970) who detected DL-diaminopimelic acid in the cell walls of *M. thermosphactum*; they thought that this gave further support for a relationship to the *Corynebacteriaceae*, but diaminopimelic acid has been detected in the walls of some lactobacilli (Ikawa (1964)).

Davis and Newton (1969) in a numerical taxonomic survey, showed that *M. thermosphactum* clustered well with *Listeria monocytogenes*, but only one named strain of each was included in their study. Davis, Fomin, Wilson and Newton (1969), however, performed a numerical taxonomic study which indicated a closer relationship between *M. thermosphactum* (two strains) and the family *Lactobacillaceae* than *Listeria monocytogenes* (12 strains). The family *Lactobacillaceae*, unfortunately, was represented solely by one unclassified heterofermentative strain of *Lactobacillus sp.*

Bacteria of the species *M. thermosphactum*, were describ-

ed by McLean and Sulzbacher (1953) as Gram positive, catalase positive rods, which ferment carbohydrates to principally lactic acid, and demonstrate low heat resistance. The species obviously differed quite considerably from M. lacticum and M. flavum, but was classified with them on the basis of its catalase activity. The DNA base ratio and lactic acid metabolism resembles Listeria and Streptococcus, but its lipid content supports its retention in the family Corynebacteriaceae.

In view of its possible resemblance to Listeria and its uncertain taxonomic position, therefore, M. thermosphactum was an obvious choice for investigation in the present study. M. flavum and M. lacticum were not included because they differed in such fundamental characters (e.g., DNA base ratio) from both M. thermosphactum and Listeria.

Lactobacillaceae.

Members of the family Lactobacillaceae Winslow et al. (1917), are described in the seventh edition of Bergey's Manual (1957), as Gram positive, lactic acid producing, catalase negative bacteria. They are short or long rods, or cocci which divide in one plane only like rods and produce chains; only occasionally are tetrads or filaments observed. Carbohydrates are essential for good growth, and are fermented to lactic acid which is possibly accompanied by volatile acids, alcohol and carbon dioxide as by-products. Oxygen requirements range from microaerophilic to strict anaerobiosis.

Of the two tribes within the family, the Lactobacilleae

includes the rod-shaped members, and the Streptococceae the coccoid forms.

Within the tribe Lactobacilleae there is one microaerophilic genus, Lactobacillus, and four anaerobic genera Eubacterium, Catenabacterium, Ramibacterium and Cillobacterium (seventh edition of Bergey's Manual (1957)). These latter, strictly anaerobic genera, were obviously of less interest for comparison with Listeria than the genus Lactobacillus, and would have posed enormous problems for inclusion in a standard numerical taxonomic survey.

Lactobacillus.

Orla-Jensen (1919) in his classical study of the lactic acid bacteria, subdivided the genus Lactobacillus into three subgenera, Thermobacterium, Betabacterium and Streptobacterium, on the basis of growth temperatures and end products of metabolism. Each subgenus contained a number of species, and the three groups are illustrated in TABLE 4, based upon Sharpe, Fryer and Smith (1966).

Briggs (1953) investigated the classification of 452 strains of lactobacilli using physiological techniques. She distinguished three groups which showed reasonable agreement with those of Orla-Jensen, and which could be further subdivided into a total of eight groups, plus 24 strains which could not be placed in any of the eight groups. Rogosa, et al. (1953) found that differences in nutritional requirements correlated well with divisions on the basis of other characteristics.

Rogosa and Sharpe (1959) reviewed the classification of the lactobacilli and accepted the subgenera of Orla-Jensen,

TABLE 4. Physiological characteristics of lactobacilli showing the three main groups and the species which they contain. (Sharpe, Fryer and Smith (1966)).

Homofermentative		Heterofermentative
carbohydrates fermented to lactic acid		carbohydrates fermented to lactic acid + volatile acid + CO_2
Thermobacterium	Streptobacterium	Betabacterium
growth at 45°C +	growth at 45°C -	
growth at 15°C -	growth at 15°C +	
L. helveticus	L. casei	L. fermenti
L. jugurt	L. plantarum	L. buchneri
L. bulgaricus		L. brevis
L. lactis		L. cellobiosus
L. acidophilus		L. viridescens
L. leichmannii		
L. delbrueckii		
L. salivarius		

as did Sharpe, Fryer and Smith (1966). Despite the fact that the names of the subgenera are no longer included in Bergey's Manual (1957), they felt that they were a useful subdivision of the genus Lactobacillus, and published a scheme for the differentiation of the species within the subgenera (Rogosa and Sharpe (1959)).

Davis (1960) also reviewed the lactobacilli, and suggested that the scheme of Rogosa and Sharpe (1959) was the most satisfactory scheme available for the whole genus. Seyfried (1968) performed a computer aided numerical taxonomic study and found that her results also supported Orla-Jensen's classification. The genus Propionibacterium was shown to be distinct from both the genus Lactobacillus and the genus Streptococcus, and her results gave no justification for the placing of the genus Lactobacillus in the family Propionibacteriaceae as suggested by Stanier, Doudoroff and Adelberg (1963).

The taxonomic subcommittee on lactobacilli and closely related organisms (1968), did much to clarify the classification of the lactobacilli, and named Type, Cotype and Neotype strains for a large proportion of the species within the genus Lactobacillus.

Serological, DNA base composition and DNA homology techniques have also been used to study the taxonomic position of the lactobacilli. The main serological studies were performed by Sharpe (1955) and Sharpe and Wheater (1957) and have resulted in the recognition of at least six serological groups. These groups resemble the physiological groupings to a certain extent, and are illustrated in TABLE 5 (Sharpe and

TABLE 5. Proposed nomenclature for serological groups of lactobacilli. (Sharpe and Wheater (1957)).

New designation	Former designation*	Species included in group
GROUP A	L. bulgaricus	L. bulgaricus L. helveticus
GROUP B	L. casei	L. casei
GROUP C	L. casei-helveticus	L. casei L. casei var rhamnosus
GROUP D	L. plantarum	L. plantarum
GROUP E	L. lactis-brevis	L. lactis subgroup L. brevis
GROUP F	L. fermenti	L. fermenti

* As given in Sharpe (1955).

Wheater (1957)). Each of the groups A, B, C, D and F included species from only one of the subgenera of Orla-Jensen (1919), A and F contained thermobacteria; B, C and D streptobacteria; but group E contained representatives of the subgenera Thermobacterium and Betabacterium. Rogosa and Sharpe (1960) have since reported that L. lactis, L. brevis, L. buchneri and L. bulgaricus all possess the E antigen, and noted that both homofermentative and heterofermentative lactobacilli are, therefore, included in serological group E.

Sharpe, Davidson and Baddiley (1964) investigated the nature of the group antigens and found that for group A the antigen was an intracellular teichoic acid, for groups D and E a wall teichoic acid, and for group F it was possibly an intra cellular teichoic acid. The walls of the bacteria in groups B, C, F and G contained no teichoic acid, and Knox (1963) demonstrated that the specific antigens of groups B and C were serologically distinct cell wall polysaccharides.

There have been many investigations of the DNA base ratios and DNA homologies of the lactobacilli, a brief review of a few of them will give an indication of the contribution which they have made to the taxonomy of the genus Lactobacillus.

Gasser and Sebald (1966) determined the DNA base compositions of representatives of the lactobacilli and found a tight group of Thermobacteria (33 to 38 moles % GC) which included L. helveticus, L. jugurt, L. salivarius and L. acidophilus. The remainder of the strains, which included members of all three subgenera, fell into a looser group, DNA base ratios ranging from 41 to 52.5 moles % GC.

Miller, Sandine and Elliker (1970), on the basis of DNA base compositions, divided the lactobacilli into three groups, see TABLE 6. Although there are similarities, their groups I, II and III did not correspond entirely with Orla-Jensen's subgenera. Miller, Sandine and Elliker (1971) studied the DNA homology of the genus Lactobacillus and the results indicated that further sub-division of their previous groups was required, see TABLE 6. Their results indicated that L. lactis and L. bulgaricus had distinctly different GC base ratios (Miller et al. (1970)). However, Dellaglio, Bottazzi and Trovatelli (1973), in their study of the DNA base composition of various thermobacteria, found that L. lactis and L. bulgaricus had similar DNA base compositions which supported the results of Simonds, Hansen and Larkshmanan (1971) who reported that the DNA base sequences of the two species were very similar.

Whilst planning the present study, an interesting, catalase positive, homofermentative bacterium was isolated from ciders by Carr and Davies (1970). The authors thought that the bacteria closely resembled members of the genus Lactobacillus, but differed sufficiently from the species of this genus listed in Bergey's Manual (1957) to warrant the proposal of a new species, L. mali.

It was decided to include in the present study most of the strains recommended as Type, Neotype or reference strains, by the Taxonomic Subcommittee on lactobacilli and closely related organisms, as well as representatives of the new species L. mali, because they share so many characters in common with Listeria.

TABLE 6. A comparison of the groups of lactobacilli based upon physiological characteristics (Orla-Jensen (1919)), DNA base ratios (Miller et al. (1970)) and DNA homologies (Miller et al. (1971)).

Groups based upon DNA base ratios.*		Species	Groups based upon DNA homologies.+	Subgenera of Orla-Jensen
GROUP	moles% GC			
I	32.4-38.3	L. helveticus	I a	Thermobacterium
		L. jugurti	I a	Thermobacterium
		L. (jugurti) bulgaricus	I a	Thermobacterium
		L. bulgaricus	I b	Thermobacterium
		L. salivarius	I b	Thermobacterium
II	42.7-48.0	L. casei	II a	Streptobacterium
		L. plantarum	II b	Streptobacterium
		L. buchneri	II c	Betabacterium
		L. brevis	II c_1	Betabacterium
		L. viridescens	II c_2	Betabacterium
III	49.0-51.9	L. fermenti	III a	Betabacterium
		L. cellobiosus	III a	Betabacterium
		L. lactis	III b	Thermobacterium
		L. leichmannii	III b	Thermobacterium
		L. delbrueckii	III b	Thermobacterium

* Miller et al. (1970).

\+ Miller et al. (1971).

o Orla-Jensen (1919).

The tribe Streptococceae, as described in the seventh edition of Bergey's Manual (1957), contains five genera, Diplococcus, Streptococcus, Pediococcus, Leuconostoc and Peptostreptococcus, and of these, the genus Streptococcus was the most obvious candidate for comparison with Listeria. Peptostreptococcus is anaerobic, Leuconostoc heterofermentative, and the other two genera are nutritionally very exacting. For a study based upon homofermentative, lactic acid bacteria which grow well on ordinary media, it was expedient to restrict representation of the Streptococceae to the genus Streptococcus.

Streptococcus.

Notable among early reviews of the streptococci are those by Andrewes and Horder (1906) and Orla-Jensen (1919). Brown (1919) published an extensive study of their haemolytic activity, but the classification system of Sherman (1937) has proved most useful. His criteria divided the genus into four groups - the enterococcus, lactic, viridans and pyogenic streptococci, see TABLE 7.

Seyfried (1968), in a computer-aided numerical taxonomic study, observed three clusters among 82 strains of streptococci, and these correlated with the pyogenic, enterococcus and lactic groups of Sherman (1937).

Many other workers have investigated or reviewed the classification of the streptococci, for example, Colman (1968), Lancefield (1969) and Sharpe, Fryer and Smith (1966); and even more have confined their studies to the enterococcus group or the streptococci of serological group D - Colwell, Mandel and Raj (1967), Deibel (1964), Drucker and Melville (1969a) and

TABLE 7. A comparison of the physiological groupings of Sherman(1937) with the serological groupings, of the genus Streptococcus.

Physiological group*	Serological group+	Species
enterococcus	D	S. faecalis
	D	S. faecalis var liquifaciens
	D	S. faecalis var zymogenes
	D	S. faecium
	D	S. durans
viridans	D	S. bovis
	D	S. equinus
	K	S. salivarius
	ungrouped	S. mitis
	"	S. thermophilus
lactic	N	S. lactis
	N	S. cremoris
pyogenic	A	S. pyogenes
	B	S. agalactiae
	C	S. equi
	C	S. equisimilis
	also E - H	

* based upon Sherman (1937).

\+ based upon Sharpe, Fryer and Smith (1966).

N.B. serological groups L to T have also been designated.

(1969b), Hartman, Reinbold and Sarawat (1966), Jones, Sackin and Sneath (1972), Raj and Colwell (1966) and Raj, Colwell and Liston(1964). In general these workers have been in agreement with the divisions of Sherman (1937).

Serological grouping has also been extremely helpful in streptococcal classification. Lancefield (1933) initiated the grouping scheme with her serological study of the haemolytic streptococci, in which groups A to E were described; there are now many more groups. The relationship between the physiological groups of Sherman (1937), and the Lancefield serological groups are illustrated in TABLE 7. Initially agreement was thought to be good between the two systems, but it was later realized that the group D antigen was found in both the enterococcus group and in some members of the viridans group.

The nature and location of several of the antigens has been determined, Hartman, Reinbold and Saraswat (1966), Jones and Shattock (1960) and Smith and Shattock (1964), see TABLE 8, based upon Hartman et al. (1966).

The DNA base compositionsof some species have been determined (see Jones and Sneath (1970)) and all fall within the range 34 to 42 moles % GC, with as yet little possibility for dividing the genus on this basis.

It is interesting that for both the genus Lactobacillus and the genus Streptococcus serological, as well as physiological characteristics, have been of major importance in classification. Representatives of the physiological and most of the serological groups of Streptococcus were chosen for inclusion in the present study because although they are

TABLE 8. The chemical nature and location of the antigenic components of some of the Lancefield serological groups. (Based upon Hartman, Reinbold and Saraswat (1966)).

Group	Group specific antigens	Chief components
A	cell wall polysaccharide	rhamnose + glucosamine
B	"	rhamnose + glucosamine
C	"	rhamnose + galactosamine
D	intracellular* teichoic acid	glucosyl-glycerophosphate
G	cell wall polysaccharide	rhamnose + galactosamine + galactose
N	intracellular* teichoic acid	galactosyl-glycerophosphate
Q	cell wall	

* intracellular means immediately exterior to the cell membrane. (Smith and Shattock (1964)).

coccoid, some strains of Listeria and Erysipelothrix can appear coccoid on first isolation, and many strains of streptococci (especially group D) can appear as elongated coccobacilli.

Brevibacteriaceae.

The family Brevibacteriaceae was constructed by Breed in the seventh edition of Bergey's Manual (1957) to provide a family for the Gram positive, non-sporing, rod-shaped bacteria displaced when the family Bacteriaceae was abolished (sixth edition of Bergey's Manual (1948)). The definition (seventh edition of Bergey's Manual (1957)) is so vague that the family can include any Gram positive, fermentative or non-fermentative, motile or non-motile, colourless, red, orange, yellow or brown rod-shaped bacteria, and there is no specification of catalase activity. There are two genera, Kurthia and Brevibacterium.

Brevibacterium.

This genus is as heterogeneous a collection of bacteria as was the family Bacteriaceae. It has been reported that Brevibacterium will not be listed as such in the next (eighth) edition of Bergey's manual, but its inclusion in the present edition, and the fact that some of the members metabolize carbohydrates fermentatively, made its representation in the present study advisable.

Kurthia.

In comparison, Kurthia is a fairly well defined, homogeneous group of bacteria, which was not an obvious choice for the present study, because it lacks fermentative carbohydrate

metabolism. The genus, however, had often been linked with M. thermosphactum on the basis of morphology, as both form relatively large colonies with deeply serrated edges on solid media (Gardner (1969)). It has been suggested that the two groups differ only in their metabolism of carbohydrates, and that they are otherwise very similar. Tests which distinguish the two groups have, however, been listed by Gardner (1969). Representatives of the species K. zopfii were included in the present study to allow yet another comparison with M. thermosphactum to be made, and to provide a possible opportunity to support the conclusions of Gardner (1969). Two other species of Kurthia, K variabilis and K. bessonii, are included in the seventh edition of Bergey's Manual (1957), but cultures of these species proved to be unobtainable.

Propionibacteriaceae.

Members of this family are characterized (seventh edition of Bergey's Manual(1957)) by the fact that they ferment carbohydrates, generally lactic acid, and in some instances polyhydroxyalcohols, to saturated aliphatic carboxylic acids. Some of these Gram positive rod shaped bacteria exhibit pleomorphism; they are generally catalase positive and are anaerobic to microaerophilic. Of the three genera, Propionibacterium, Butyribacterium and Zymobacterium, the former adapts quite readily to growth under aerobic conditions, and was, therefore, of greater interest for the present study.

Propionibacterium.

Members of the genus Propionibacterium produce propionic acid as the main fermentation product, with acetic acid and

traces of succinic acid and carbon dioxide. They are very pleomorphic, which possibly suggests a relationship to the family Corynebacteriaceae. As mentioned earlier, Seyfried (1968) could find no justification for placing the genus Lactobacillus in the family Propionibacteriaceae, as Stanier, Doudoroff and Adelberg (1963) had suggested.

Micrococcaceae.

This family is defined in the seventh edition of Bergey's Manual (1957) as a group of Gram positive, non-spore-forming, catalase positive cocci, many of which produce non-water-soluble yellow, orange, pink or red pigments. There is a range from saprophytic to parasitic, sometimes pathogenic, and the skin and skin glands are the most frequent habitat. Oxygen requirements range from aerobic to anaerobic. There are six genera listed in the seventh edition of Bergey's Manual (1957), Micrococcus, Staphlyococcus, Gaffkya, Sarcina, Methanococcus and Peptococcus. The latter two genera are strictly anaerobic, and do not, therefore, fall within the realm of the present study. The generic name Gaffkya, has been rejected by the Judicial Commission (1971) and the type species of the genus Sarcina, S. lutea, has been removed by Baird-Parker (1963) to the genus Micrococcus, and renamed M. luteus. It was, therefore, doubtful whether the inclusion of representatives of the genera Gaffkya and Sarcina was justifiable. Since the family was mainly of interest as a marker group, a few members of the more well-defined genera, Micrococcus and Staphylococcus, provided sufficient representation.

The taxonomic position of the genera Staphylococcus and Micrococcus has been discussed by many workers, for example, Baird-Parker (1963), Jones, Deibel and Niven (1963) and Jones and Niven (1964), and it is generally accepted that those strains which are able to grow anaerobically in a complex medium containing glucose belong to the genus Staphylococcus, whereas those which do not grow belong to the genus Micrococcus (Evans, Bradford and Niven (1955)).

There were a few unusual organisms, of which the taxonomic position was unclear, which were also chosen for inclusion in the present study. Two atypical strains of Arthrobacter sp., which had been isolated from throat swabs by Dr. B. Brzin, were included because of their possible pathogenicity, and because of a superficial morphological resemblance to an odd strain, G 9, received as a strain of Listeria sp. Other organisms which were included were three strains of Bacterium eurydice, three strains of Gemella haemolysans and one strain received as Neisseria haemolysans.

Bacterium eurydice.

This bacterium was isolated as a secondary invader of the european foulbrood disease of honey bees (White (1920) and Bailey (1960)) and from apparently healthy honey bees (Bailey (1963)). It is refered to as Achromobacter eurydice in the seventh edition of Bergey's Manual (1957). Bacillus pluton or Streptococcus pluton was also isolated from cases of european foulbrood and was believed by White (1920) and Bailey (1960) to be the causal agent of the disease. Burri (1943) suggested that B. eurydice and S. pluton were different morphological forms of the same species, and that the

rods of B. eurydice, under certain conditions, gave rise to the cocci of S. pluton. Both species exhibit better growth under anaerobic conditions, but S. pluton utilizes glucose and fructose with no acid production, while B. eurydice ferments carbohydrates. S. pluton is weakly catalase positive, but B. eurydice is catalase negative, and Bailey (1957) reported a potassium dependence for S. pluton which is not exhibited by B. eurydice. It, therefore, appeared that these two species are, in fact, distinct from each other.

In view of its fermentative carbohydrate metabolism, and the fact that so little was known about it, Bacterium eurydice was an interesting species for comparison with the lactic acid bacteria.

Gemella haemolysans.

This species was first described by Thjøtta and Bøe in 1938 and named Neisseria haemolysans. It was described as a Gram negative coccoidal bacterium which resembled N. sicca in its fermentation reactions, but differed from it because it was haemolytic. The species was listed in the seventh edition of Bergey's Manual (1957).

In 1961 Berger reinvestigated the species and found that the strains were catalase, oxidase and peroxidase negative, characteristics which he thought resembled less Neisseria than, for example, the streptococci. He, therefore, proposed that a new monotypic genus, Gemella haemolysans, be created for these bacteria so that they could be removed from the genus Neisseria, and that Gemella should be included in the family Neisseriaceae together with the genera Neisseria and Veillonella.

Yamakawa and Ueta (1964) investigated the fatty acid and carbohydrate compositions of Neisseria species, and found that N. haemolysans differed from the other Neisseria in both of these characteristics. Reyn, Birch-Andersen and Lapage (1966) showed, on the basis of an electron microscope study, that N. haemolysans possessed a cell wall, internal structure and mode of division resembling that of Gram positive bacteria, characteristics which they thought supported the acceptance of the generic name Gemella, to separate these bacteria from the other members of the genus Neisseria. Reyn, Birch-Andersen and Berger (1970) further investigated the group and reported that the DNA base ratio was 33.5 moles % GC, compared with values in the region of 50 moles % GC for the other members of the genus Neisseria. These workers and Reyn (1969) and (1970) suggested that Gemella haemolysans was not related to the Neisseriaceae but should be assigned to a family of Gram positive bacteria, possibly the Lactobacillaceae in the tribe Streptococceae. Clearly it was important to include representatives of this group in the present study.

As stated earlier the aim of the work reported here was to investigate the taxonomic position of Listeria. The production of mainly lactic acid by Listeria suggests a relationship to the family Lactobacillaceae (Miller and Silverman (1959)), but its weak catalase production has so far prevented its inclusion in the family. Erysipelothrix, however, produces lactic acid, does not produce catalase, or contain cytochromes, and like Listeria, fails to exhibit "coryneform" morphology and is, therefore, a strong candidate for inclusion in the family Lactobacillaceae. Microbacterium thermos-

phactum physiologically closely resembles Listeria, but shows a strong catalase reaction. In view of reports by Dacre and Sharpe (1956), Vaňková(1957) and Whittenbury (1964) that weak catalase activity has been detected in bacteria already placed in the family Lactobacillaceae, it appears that the family should be redefined, in which case, Listeria and M. thermosphactum would be strong contenders for transfer into the Lactobacillaceae.

In choosing bacteria for the present study of the taxonomic position of Listeria, representatives of the following genera were included, from a variety of sources, and including the type strains whenever possible - Bacterium (3), Brevibacterium (7), Cellulomonas (6), Corynebacterium (9), Erysipelothrix (13), Gemella (4), Kurthia (10), Lactobacillus (25), Listeria (48), Microbacterium (25), Micrococcus (4), Propionibacterium (4), Staphylococcus (4), Streptococcus (28) and two unidentified strains from throat swabs.

Due to the limitations imposed by the computer program, and time, no strictly anaerobic strains were included, nor any heterofermentative strains other than those within the genus Lactobacillus. There is undoubtedly a need to study the relationships of the heterofermentative strains, from genera such as Leuconostoc, but the principle objective of the present study was to investigate the relationships of the homofermentative lactic acid genus, Listeria.

It was decided to study the strains of Listeria and the chosen comparative organisms by two techniques, computer-aided numerical taxonomy and serology.

METHODS.

METHODS

I. Strains and Maintenance.

All bacteria [Appendix I] were maintained in petri dishes on Basal Medium agar (hereafter referred to as BM agar) [see Appendix II for constitution and section (a) below for a description of its development]. The lactobacilli and streptococci, which tended to exhibit good growth, but limited survival on BM agar, were subcultured twice weekly, all other bacteria once a week. Also, on receipt, all bacteria were subcultured, tested for purity and lyophilised using a Speedivac centrifugal freeze-dryer (Model 5PS Edwards High Vacuum Ltd.). The ampoules obtained, having been tested for survival and purity, were stored as a reserve source of the bacteria.

a) Choice of Basal Media.

The diversity of the taxa chosen necessitated the development of a basal medium which allowed good growth of all the strains. The lactobacilli were the most demanding nutritionally, and therefore, the media of DeMan, Rogosa and Sharpe (DeMan, Rogosa and Sharpe, 1960) (Oxoid M.R.S. agar CM361, and M.R.S. broth CM359), developed by these workers for the growth of lactobacilli, were chosen as a possible basis.

Initially, growth of representatives from each taxon, on M.R.S. agar and in M.R.S. broth, was investigated. However, as some bacteria (e.g., Streptococcus pyogenes G120) showed

limited or no growth on M.R.S. agar or in M.R.S. broth, it was decided to study the effect of omitting the acetate and, or citrate. Growth of the lactobacilli was not significantly reduced by omitting the acetate or citrate, some bacteria grew better with one of the single omissions (e.g., S. pyogenes G120 on omission of acetate, Microbacterium thermosphactum G86 on omission of citrate), but all exhibited good growth in the absence of both the citrate and the acetate.

The modified M.R.S. agar and M.R.S. broth were both tested as growth media for the entire collection and found to be suitable. These modifications of M.R.S. agar and M.R.S. broth will hereafter be referred to as BM agar, and Basal Medium broth (BM broth), respectively.

A further basal medium was found to be necessary for certain tests, as Lab Lemco (Oxoid L29) and Yeast Extract (Oxoid L21), two of the constituents of BM agar and BM broth, contain traces of trehalose which can act as a carbon source. This had obvious implications for sugar fermentation tests, tests of ability to use various carbon sources, and certain hydrolysis reactions. A "Sugar" Basal Medium (hereafter referred to as SBM) was therefore developed, in which Lab Lemco was completely omitted, and Yeast Extract was used at a reduced level of 1g. per litre. When brom-cresol purple was added to this medium, none of the strains showed any detectable acid production, which demonstrated its suitability as a basal medium for fermentation tests etc.

b) Choice of Incubation Temperatures

It was not possible to use a single incubation temperature for the survey, because there was no one temperature at which all of the strains would grow. Microbacterium thermosphactum, for example, will not grow at 35°C, whereas certain lactobacilli and streptococci grow barely, or not at all, at 30°C. Since the majority of the strains grew well at 35°C, this was chosen as the main incubation temperature.

The strains of M. thermosphactum, together with a representative selection of 46 strains from the other genera, were incubated at 30°C. These latter strains were grown at 30°C and 35°C so that the variation produced by altering the incubation temperature could be found, thus giving an indication of the effect on clustering, similarity levels, etc., of using two incubation temperatures in the survey.

II. Bacteriological Test Methods.

a) Morphological.

These tests were used to obtain the results for characters 1-34 in TABLE 9. Blood Agar [see Appendix II] was used for the colonial morphology and Gram stains, all results being read at 1 and 4 days. The haemolytic ability (alpha or beta haemolysis) of the strain was recorded after 1 and 2 days. the cultural characteristics in a liquid medium were studied in BM broth after incubation for 1 and 4 days. Motility and tumbling were investigated (by the hanging drop technique) using BM broth incubated for 24 hours.

TABLE 9. List of Tests Used in the Numerical Taxonomic Survey.

Test.		Times of Reading (days).	Reading Used for Computer.	Coding for the Computer.
A. Morphological Characters.				
1 Surface	smooth/rough	1 and 4	4	0/1
2 Margin	entire/serrated	"	4	0/1
3 Elevation	low convex	"	4	0/1
4 "	convex	"	4	0/1
5 "	"fried egg"	"	4	0/1
6 Consistency	watery	"	4	0/1
7 "	butyrous	"	4	0/1
8 "	viscous	"	4	0/1
9 "	"bitty"	"	4	0/1
10 Translucent colony		"	4	0/1
11 Giving an homogeneous emulsion		"	4	0/1

TABLE 9. (continued.)

Tests.		Times of Reading (days).	Reading Used for Computer.	Coding for the Computer.
12 Pigmentation	yellow-cream	1 and 4	4	0/1
13 "	pink	"	4	0/1
14 Gram reaction	positive	"	4	0/1
15 Cell Form	long rods	"	4	0/1
16 "	short rods	"	4	0/1
17 "	coccobacilli	"	4	0/1
18 "	cocci	"	4	0/1
19 "	filaments	"	4	0/1
20 "	tendency to chain formation	"	4	0/1
21 Adherence		"	4	0/1
22 Haemolysis	alpha	1 and 2	2	0/1
23 "	beta	"	2	0/1

TABLE 9. (continued.)

Tests.		Times of Reading (days).	Reading Used for Computer.	Coding for the Computer.
24 Colony diameter	less than 1mm/ 1-3mm/3mm or above	1 and 4	4	0/1/2
25 Pleomorphism		"	4	0/1
26 Pellet	present	"	4	0/1
27 "	stringy	"	4	0/1
28 "	granular	"	4	0/1
29 "	flocculent	"	4	0/1
30 Pellicle/Ring	neither/ring/ pellicle	"	4	0/1/2
31 Easy resuspension of the pellet		"	4	0/1
32 Beaded or Granular Gram Staining		"	4	0/1
33 Motility		1	1	0/1
34 Tumbling in a Hanging Drop		1	1	0/1

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Coding for the Computer
B. Biochemical and Physiological Characters.				
35	Cellulose hydrolysis	70	70	0/1
36	Tween 20 "	1,4,7,10,14,21	21	0/1
37	" 40 "	"	"	0/1
38	" 60 "	"	"	0/1
39	" 80 "	"	"	0/1
40	Tyrosine "	"	"	0/1
41	Xanthine "	"	"	0/1
42	Growth at 5°C	3,7,14,21	"	0/1/2
43	" 10°C	1,3,7,14	14	0/1/2
44	" 20°C	"	"	0/1/2
45	" 30°C	1,3,7	7	0/1/2

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Coding for the Computer
46	Growth at 35°C	1,3,7	7	0/1/2
47	" 45°C	"	"	0/1/2
48	" 50°C	"	"	0/1/2
49	Gluconate oxidation	8	8	0/1
50	Indole production from tryptone	16	16	0/1
51	Lecithinase activity	1,2,8	8	0/1
52	Methyl Red Test	3 and 7	7	0/1
53	Voges-Proskauer Test	7	7	0/1
54	Inhibition by thallous acetate			
	0.01%	1 and 2	2	0/1/2
55	" " 0.02%	"	"	0/1/2
56	" " 0.03%	"	"	0/1/2

TABLE 9. (continued).

	Tests		Times of Reading (days)	Reading used for Computer	Coding for the Computer
57	Catalase activity		1	1	0/1
58	Oxidase "		"	"	0/1
59	Growth under hydrogen		5	5	0/1
60	Inhibition by sodium chloride	6.5%	1,2,6	6	0/1/2
61	" "	10.0%	"	"	0/1/2
62	Reduction of potassium tellurite	0.01 %	1 and 2	2	0/1
63	" "	0.025%	"	"	0/1
64	" "	0.05 %	"	"	0/1
65	Inhibition by tetrazolium	0.01 %	"	"	0/1/2
66	Reduction of "	0.01 %	"	"	0/1
67	Inhibition by "	0.1 %	"	"	0/1/2

TABLE 9. (continued).

	Tests		Times of Reading (days)	Reading used for Computer	Coding for the Computer
68	Reduction of tetrazollium	0.1 %	1 and 2	2	0/1
69	Inhibition by potassium thiocyanate				
		2.5 %	"	"	0/1/2
70	" "	3.7 %	"	"	0/1/2
71	" "	4.5 %	"	"	0/1/2
72	" sodium azide	0.01 %	"	"	0/1/2
73	" "	0.02 %	"	"	0/1/2
74	" "	0.05 %	"	"	0/1/2
75	Ammonia production from peptone		1,2,3.4	4	0/1
76	Hippurate hydrolysis		8	8	0/1
77	Arginine deamination (oxidative)		2 and 5	5	0/1
78	" " (fermentative)		"	"	0/1

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Coding for the Computer
79	Aesculin hydrolysis	1 and 5	5	0/1
80	Sulphatase activity	6	6	0/1
81	Phosphatase "	"	"	0/1
82	DNase "	"	"	0/1
83	RNase "	"	"	0/1
84	Gas production from glucose	1,2,5,7	7	0/1
85	Urease activity	1,4,7,11,14,21	21	0/1
86	Hydrogen sulphide production	"	"	0/1
87	Inhibition by Penicillin	1 and 2	2	0/1/2
88	" Streptomycin	"	"	0/1/2
89	" Chloramphenicol	"	"	0/1/2
90	" Chlortetracycline	"	"	0/1/2

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Coding for the Computer
91	Inhibition by Oxytetracycline	1 and 2	2	0/1/2
92	" Erythromycin	"	"	0/1/2
93	" Tetracycline	"	"	0/1/2
94	" Sulphanilamide	"	"	0/1/2
95	" Novobiocin	"	"	0/1/2
96	" Oleandomycin	"	"	0/1/2
97	" Naladixic acid	"	"	0/1/2
98	Stimulation by Sulphanilamide	"	"	0/1
99	Survival at 60°C for 15 min.	"	"	0/1
100	Nitrate reduction to nitrite	2 and 8	8	0/1
101	" " nitrogen	"	"	0/1
102	Inhibition by nitrite	"	"	0/1

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Coding for the Computer
103	Starch hydrolysis	15	15	0/1
104	Casein "	1,2,5,8,11,15	15	0/1
105	Glucose breakdown (oxidative)	1,2,4,5,8,11,14	14	0/1
106	" " (fermentative)	"	"	0/1
107	Acid from D-inulin	1,2,3,4	4	0/1/2
108	" D-inositol	"	"	0/1/2
109	" D-dulcitol	"	"	0/1/2
110	" D-lactose	"	"	0/1/2
111	" α-methyl-D-glucoside	"	"	0/1/2
112	" D-melibose	"	"	0/1/2
113	" salicin	"	"	0/1/2
114	" adonitol	"	"	0/1/2

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Coding for the Computer
115	Acid from D-arabinose	1,2,3,4	4	0/1/2
116	" D-mannitol	"	"	0/1/2
117	" glycerol	"	"	0/1/2
118	" D-maltose	"	"	0/1/2
119	" rhamnose	"	"	0/1/2
120	" D-galactose	"	"	0/1/2
121	" D-sucrose	"	"	0/1/2
122	" D-sorbitol	"	"	0/1/2
123	" D-fructose	"	"	0/1/2
124	" xylose	"	"	0/1/2
125	" trehalose	"	"	0/1/2
126	" D-raffinose	"	"	0/1/2

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Coding for the Computer
127	Acid from melezitose	1,2,3,4	4	0/1/2
128	" D-cellobiose	"	"	0/1/2
129	Litmus Milk acid production	1,2,5,9	9	0/1
130	" reduction	"	"	0/1
131	" clot production	"	"	0/1
132	" alkali "	"	"	0/1
133	" alkaline digestion	"	"	0/1
134	Utilization of pyruvate	5	5	0/1/2
135	" acetate	"	"	0/1/2
136	" propionate	"	"	0/1/2
137	" citrate	"	"	0/1/2
138	" malate	"	"	0/1/2

TABLE 9. (continued).

	Tests	Times of Reading (days)	Reading used for Computer	Code for the Computer
139	Utilization of malonate	5	5	0/1/2
140	" α-keto-glutarate	"	"	0/1/2
141	" salicylate	"	"	0/1/2
142	" glucose	"	"	0/1/2
143	slime production on sucrose	"	"	0/1

b) Biochemical and Physiological.

All test media were inoculated using 48 hour cultures of the strains on BM agar, and incubated at their chosen temperature, unless otherwise stated. The tests, times of reading and coding for the computer are listed in TABLE 9, details of the test media and test reagents are given in Appendix II. An uninoculated control was always included, and when divided dishes were employed the multipoint inoculator of Sneath and Stevens, (1967) was used for inoculation and subculture.

Aesculin hydrolysis was observed in aesculin broth, as a blackening and loss of sheen 1 and 5 days after inoculation.

Ammonia production from peptone was studied in divided dishes using solid SBM plus brom-cresol purple (0.003%w/v). A deep purple colouration indicated ammonia production.

Antibiotic sensitivity. The organisms were grown in 7-8ml quantities of BM broth for 24 hours. These broths were then used to seed three BM agar plates per organism; when dry, high potency Sentest tablets (Evans Medical, Speke, Liverpool) were dispensed onto the lawns using a special applicator. The plates were observed after 1 and 2 days for partial and complete zones of inhibition, and also for stimulation in the case of sulphanilamide. The antibiotics are listed in TABLE 9 (characters 87-97).

Arginine deamination was observed as production of an alkaline pH which caused the neutral red indicator to change from a straw colour to pink. The test was performed aerobically and anaerobically, and readings were taken after 2 and 5 days incubation.

Carbon sources. SBM broths containing 1%w/v of each of the

carbon sources (characters 134-142 in TABLE 9) and control tubes containing SBM broth only, were inoculated for each bacterium and incubated for 5 days. The absorbance at 540nm. was recorded using a Bausch and Lomb Spectronic 20 spectrophotometer, and a carbon source was considered to have been utilised if the absorbance of the particular tube was at least twice that of the control tube.

Casein hydrolysis was tested by streaking Casein agar plates with each bacterium, and observing for zones of clearing and, or, precipitation after 1,2,5,8,11 and 15 days.

Catalase activity was tested by placing a drop of 20 volume hydrogen peroxide on 24 hour cultures on BM agar, and observing for the liberation of bubbles of oxygen.

Cellulose hydrolysis was tested using BM broth minus the glucose, to which strips of Whatman No. 1 filter paper were added such that the paper was only half submerged in the broth. The tubes were observed up to 70 days after inoculation for breakdown of the paper.

Deoxyribonuclease activity was investigated using DNase agar inoculated with a single streak of each bacterium. Observations for zones of hydrolysis were made after 6 days incubation, and for uncertain results the plates were flooded with 1N HCl for a few minutes, to facilitate detection of any zones of clearing by increasing the opacity of unhydrolysed areas of DNA.

Gas production from glucose. Tubes of Glucose broth were inoculated, and observations were made for accumulation of gas (CO_2) in the Durham tubes after incubation for 1,2,5 and 7 days.

Gluconate hydrolysis was tested using SBM broth plus 1%w/v gluconate. After incubation for 8 days a Clinitest tablet (Ames Company, Slough, Bucks.) for the detection of glucose was added to 1ml. of the broth. The production of a blue colouration indicated the absence of glucose, and any shade of orange, the presence of glucose.

Glucose breakdown. O and F test media, one tube with a paraffin seal and one without, were inoculated with each bacterium by stabbing a straight wire to the bottom of the tube. Observations for acid production were made after incubation for 1,2,4,5,8,11 and 14 days.

Growth under an atmosphere of hydrogen was tested by inoculating two tubes of BM broth for each organism, one was placed in an anaerobe jar under hydrogen, and the other incubated normally. The latter tube was used as a check of viability, and after 5 days the presence or absence of growth under hydrogen was recorded.

Hippurate hydrolysis. Hippurate broths were incubated for 7 days, if the growth had settled, leaving the broth clear, two 1ml. samples were taken from each bottle, but if the broth was turbid, they were centrifuged before the samples were removed. The first test reagent was 12%w/v ferric chloride to which 2.5ml. conc. hydrochloric acid had been added per 1., and the second was 50%v/v sulphuric acid. It is necessary to standardise each batch of media, by adding the ferric chloride solution dropwise until the initial orange-brown precipitate just dissolves. Normally 1ml. of the ferric chloride solution is required per 1ml. of broth. This volume was then added to one tube of each pair, and left to stand, with occasional

shaking, for 30 to 60 min. The presence of a precipitate at the end of this period indicated the hydrolysis of hippurate. Any uncertain results were checked by the addition of 1ml. of 50% sulphuric acid to the second 1ml. sample of that particular bacterium, a white precipitate indicated hippurate hydrolysis. This latter method is more sensitive, but less reproducible, hence the use of both methods for detecting hippurate hydrolysis.

Hydrogen sulphide production. The urea slopes were used for this test, on inoculation, strips of lead acetate paper were placed inside the tops of the tubes. Hydrogen sulphide production was indicated by a blackening of the paper due to lead sulphide production. The results were recorded after 1, 4,7,11,14 and 21 days incubation.

Indole production from tryptone was tested by inoculating 7ml. quantities of tryptone broth. After incubation for 16 days 0.5ml. of Kovác's reagent was added, followed by a thorough mixing. The production of a pink colouration in the upper alcohol layer indicated the production of indole.

Lecithinase activity was tested using BM agar with the glucose concentration reduced to 0.1%w/v. After autoclaving, when the medium had cooled to below 60°C, sterile lecithin (Oxoid SR47) was added to a concentration of 1%. The plates were streaked with each organism and observed for zones of clearing and, or, precipitation after 1,2, and 8 days of incubation.

Litmus milk. Observations were made for acid production (conversion of the litmus from purple to pink), reduction (a loss of colour due reduction of the indicator), clotting

(solidification of the milk), alkali production (the production of a deep purple colouration), and digestion (resulting in a very watery consistency) after 1,2,5 and 9 days incubation.

Methyl Red Test. MRVP medium was tested after 3 and 7 days incubation, by aseptically removing 3 drops of broth, placing it on a spotting tile and adding 1 drop of methyl red indicator (0.04g. methyl red dissolved in 40ml. of methanol to which 100ml of distilled water was then added). The production of a red or orange colour indicated a positive reaction, i.e., the production of a pH of approximately 4.5 or below in a buffered broth.

Nitrate reduction. Nitrate broths were tested after incubation for 2 and 8 days. The Durham tubes were observed for the presence of gas (nitrogen), and 1ml. samples of broth were removed aseptically to a divided dish where 1ml. of each of the test reagents, Nitrate A and Nitrate B, were added. The development of a pink to dark reddish-brown colouration indicated the presence of nitrite in the broth. Zinc was then added to all samples giving a negative result to check for the presence of residual nitrate.

Nitrite sensitivity was tested using nitrite broth and a BM broth control, growth was compared in the tubes 2 and 8 days after inoculation.

Oxidase activity. A small amount of growth from a 24 hour culture on BM agar was spotted onto a filter paper soaked in a fresh solution of n'n'n'n' tetramethyl paraphenylene diamine dihydrochloride(1%w/v). A platinum loop was used as other metals can result in the production of a false positive

reaction. A positive result was recorded if a purple colouration developed within 30 secs.

Phosphatase activity was investigated by streaking the test organisms onto Phosphatase agar. After incubation for 6 days 1 ml. of concentrated ammonia was placed in the lids of the inverted dishes. The production of a pink colouration around the streak indicated that the phenolphthalein diphosphate had been split to yield the monophosphate which appears pink at an alkaline pH.

Potassium tellurite reduction. Divided dishes containing Potassium tellurite agar were inoculated and observed after 1 and 2 days incubation for reduction of potassium tellurite, as indicated by a blackening of the agar and, or, bacterial growth.

Potassium thiocyanate sensitivity was tested by inoculating divided dishes containing Potassium thiocyanate agar (2.5%, 3.75% and 4.5%w/v) and BM agar, and comparing the growth after 1 and 2 days incubation.

Ribonuclease activity was tested in the same manner as DNase activity except that RNA (0.2%w/v) replaced the DNA in the DNase agar.

Slime production on Sucrose was investigated by culturing the bacteria in divided dishes containing SBM agar to which 1%w/v sucrose (filter sterilised) had been added after autoclaving. The production of slime could be observed as a visible change in the colonial morphology, readings at 1 and 2days.

Sodium azide sensitivity. Growth after 1 and 2 days incubation in divided dishes containing Sodium azide agar (0.01%, 0.02% and 0.05%w/v) was compared with that on BM agar.

Sodium chloride sensitivity. Tubes containing 5ml. quantities of BM broth plus 6.5% and 10%w/v sodium chloride were inoculated together with BM broth control tubes, and observed after 1,2 and 7 days incubation. The occurrence of inhibition by sodium chloride was determined by comparison of the amount of growth in the three sets of tubes.

Starch hydrolysis was tested by streaking each bacterium onto Starch agar and incubating for 15 days. A little growth was then scraped away from each streak, the plates flooded with iodine and observed for zones of hydrolysis.

Sugar fermentation tests. SBM agar plus brom-cresol purple indicator (0.003%w/v) was autoclaved and allowed to cool to approximately 60°C. Filter sterilised solutions of the sugars to be tested (characters 107 to 128 in TABLE 9) were added to a final concentration of 1%w/v and the medium was dispensed into divided dishes, inoculated and observed for acid production after 1,2,3 and 4 days incubation.

Sulphatase activity was tested as for phosphatase activity, but 1%w/v phenolphthalein disulphate replaced the diphosphate in the Phosphatase agar.

Temperature range was tested as ability to grow in BM broth at 5°C, 10°C, 20°C, 30°C, 35°C, 45°C and 50°C (to an accuracy of $\pm$ 1°C). The tubes were read up to 21 days for 5°C, 14 days for 10°C and 20°C and 7 days for all other temperatures. Ability to withstand 60°C for 15 mins. was tested by placing 18hour broth cultures at 60°C, in a water-bath, for 15 mins. The tubes were immediately cooled in cold water and two drops from each tube plated onto BM agar. Any growth on these plates was observed after 1 and 2 days incubation.

Tetrazolium reduction and sensitivity. Cultures grown on tetrazolium agar in divided dishes were observed after 1 and 2 days incubation for pink colouration due to reduction of tetrazolium, and for degree of growth compared with that in simultaneously inoculated dishes of SBM agar plus 2%w/v glucose.

Thallous acetate sensitivity. Thallous acetate agar (0.01%, 0.02% and 0.03%w/v) in divided dishes was inoculated simultaneously with a set of BM agar dishes, and sensitivity was established by comparison of the degree of growth after 1 and 2 days incubation.

Tween hydrolysis was investigated by streaking each bacterium onto Tween agar containing Tweens 20, 40, 60 and 80, and observing for zones of hydrolysis (clearing and, or, precipitation) after 1,4,7,10,14 and 21 days incubation.

Tyrosine hydrolysis. Single streaks of each bacterium were made on Tyrosine agar and observed for zones of clearing or precipitation after incubation for 1,4,7,10,14 and 21 days.

Urease activity was investigated by inoculating Urea agar slopes, and observing them after 1,4,7,11,14 and 21 days incubation for the pink colouration, typical of an alkaline pH, produced as the result of ammonia production.

Voges-Proskauer test. The MRVP broths were tested 7 days after inoculation, when samples for the MR test had already been removed. 1ml. of 5%w/v α-naphthol solution, followed by 1ml. of 40%w/v potassium hydroxide solution were added to each universal bottle and mixed vigourously. The tests were left to stand with occasional shaking for half an hour, and a positive reaction was indicated by a deep red colouration.

Xanthine hydrolysis was tested by observing single streaks of each strain on Xanthine agar, for zones of clearing, or precipitation, or both after 1,4,7,10,14 and 21 days incubation.

III. Computer Methods.

The data obtained from the tests included in the numerical taxonomic survey were subjected to numerical taxonomic analysis using an ICL 4130 computer. Clustering by the Unweighted Pair Group Method (UPGM) and Single Linkage Method (SLM) (Sneath and Sokal (1973)) and analysis of the various clusters was performed using the programs routinely employed in the Medical Research Council, Microbial Systematics Unit, written by M.J.Sackin and J.Campbell-Kelly. In addition, a form of n-dimensional clustering was performed using the programs of J.W.Carmichael, TAXON and TAXMAP (Carmichael, Julius and Martin (1965), Carmichael, George and Julius (1968) and Carmichael and Sneath(1969)).

For reviews of the use of computers in taxonomy see Sneath (1964) and (1971), Gower (1969) and Sneath and Sokal (1973).

The results were coded as follows: negative results were coded '0' and for most characters positive results were coded '1'. With some tests, however, three character states could be recognised: negative- '0', weak positive- '1' and strong positive- '2', e.g., strong and weak acid production from carbohydrates and complete and partial inhibition by antibiotics. These latter characters were multistate as opposed to the simple binary or two-state characters first mentioned.

In the comparison of results for a two-state character, a pair of OTUs (Operational Taxonomic Units - in this instance bacterial strains) were either completely identical or completely dissimilar. With multistate characters, '0' and '1' or '1' and '2' showed partial similarity expressed as 0.5 or 50% similarity, and '0' and '2' were considered completely dissimilar. The similarities from individual characters were then combined to give the overall similarities, using the coefficient of Gower (1969), S_{GOWER}, for that pair of OTUs. This coefficient includes negative matches and is very similar to the commonly used simple matching coefficient, but permits the inclusion of a 50% similarity for a character coded into three states as explained above.

Initially the data was punched onto computer cards and then read onto magnetic tape by the program ITBNTOMT (integer t by n to magnetic tape). The program ITBNCLST (integer t by n cluster) was then used to read the data from magnetic tape and to perform numerical taxonomy on it. At this stage characters, or OTUs, or both could be deleted. A similarity matrix was computed by comparing each OTU with every other OTU and calculating the Gower similarity coefficients. Other similarity coefficients were available, but only S_{GOWER} permitted the use of multistate characters. Clusters could then be formed by one of three options, WPGM (Weighted Pair Group Method), UPGM (Unweighted Pair Group Method) or SLM (Single Linkage Method), of which the two latter methods were employed. A dendrogram was then printed, and there was an option enabling ITBNCLST to punch a coded form of the dendrogram onto paper tape for input into the program DENDPROG

which then plotted the dendrogram on a digital plotter. ITBNCLST also printed a similarity frequency scatter diagram and cophenetic correlation statistics between the similarity matrix and dendrogram. These statistics give an indication of the distortion incurred in the conversion of the similarity matrix to the dendrogram; a high cophenetic correlation indicates low distortion. The sorted similarity matrix (i.e. with the OTUs in the same order as along the tips of the corresponding dendrogram) was then printed, both to three significant figures and to one digit. The latter was useful for a quick indication of the groups and for the production of shaded similarity matrices.

The Carmichael programs, TAXON and TAXMAP were also used, but proved of little additional value with the present data. Results have usually (Carmichael et al. (1965) and (1968) and Carmichael and Sneath (1969)) proved amenable to two dimensional diagrammatic representation, or the production of three dimensional models; but since many of the species in the present study (e.g., most Streptococcus and Lactobacillus species) were only represented by a single strain, many single-member clusters were produced. Diagrammatic representation was, therefore, very difficult and since it afforded no further information than the programs ITBNCLST and IGROUPS, it was not considered worthwhile to plot out the relationships in detail.

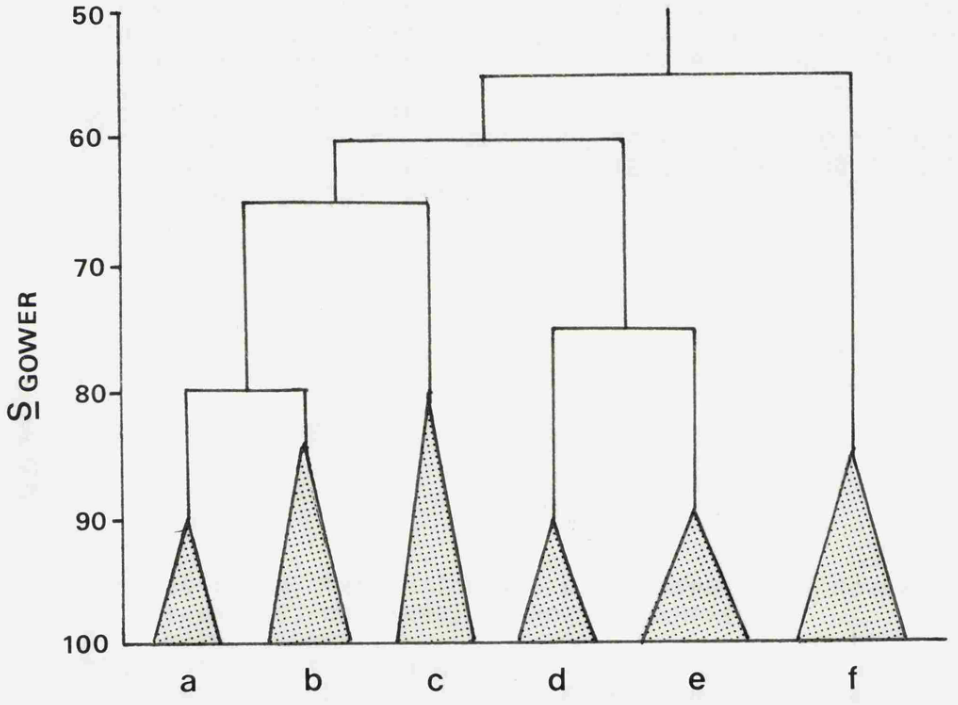
The program IGROUPS (Integer groups) printed character value statistics on specified groups of OTUs chosen from an integer t by n matrix on magnetic tape. The mean similarity of each OTU to all the groups was calculated and printed;

mean inter- and intra-group similarities, their variances and standard deviations were also given. The program had three options for computing character value statistics, but only two of these were of use for this data. The percentage of positive results for the binary characters was printed for each group in turn (in descending order of the percentage of positive results) together with the percentage of positive results for each character and all OTUs. The other appropriate option applied to both binary and multistate characters. The numbers of '0', '1' and '2' results within each group were printed for each character in turn. This second option proved very useful in the determination of key characters whose different states corresponded closely with the different groups formed in the numerical taxonomic analysis. TABLE I in Appendix III was constructed from this sort of computer output, and TABLE 15 in the results section was produced by comparison of the results shown in TABLE I (III). Also, a series of runs were performed in which key characters were obtained for groups formed at a series of selected levels of similarity. For example, in the hypothetical dendrogram in Figure 1 the results for the following groups could be compared: a + b + c + d + e with f; a + b + c / d + e; a + b / c; a / b and d / e, in an attempt to determine discriminating characters for the various groups. TABLE 16 in the results section was produced in this way.

The inter- and intra-group similarities were used to produce a three-dimensional model of the taxonomic relationship between the main groups of OTUs formed in the study. A photograph of this model is shown in the results section

(Figure 7.).

Figure 1. Hypothetical dendrogram to illustrate the use of one of the options in the program IGROUPS.



It was thought desirable to include the coded data matrix in the thesis for completeness. The program PRINTRES (print results) was therefore written, with the kind assistance of M. J. Sackin, to print the data matrix in sections of suitable size for a sheet of A4 paper. This greatly facilitated the inclusion of the results, in Appendix III , and the program is listed below.

Computer print out of the program PRINTRES.

```
  1   PRINTRES;
  2   "BEGIN" "INTEGER" TMAX, NMAX, TYPE, T, N, IDENT, J;
  3      "BOOLEAN" RNAMPRES, CNAMPRES, Q, ERROR;
  4      "INTEGER" "ARRAY" TITLE [1:65];
  5   "COMMENT" READS INTEGER TBYN FROM CARDS PRINTS IN TABLE FORM- 50 OTUS
  6         BY 18 CHARS PER PAGE;
  7      J:=1;
  8      INSTRING (TITLE,J);
  9      J:=1;
 10      "PRINT" ''F'';
 11      OUTSTRING (TITLE,J);
 12      "READ" TMAX, NMAX, TYPE, RNAMPRES, CNAMPRES;
 13      "PRINT" PREFIX(''L3'TMAX='), LEADZERO ('0'), TMAX, PREFIX (', NMAX='),
 14         NMAX, PREFIX(',TYPE='),  TYPE, ',RNAMPRES=', RNAMPRES,
 15         ', CNAMPRES=', CNAMPRES;
 16      "BEGIN" "INTEGER" "ARRAY" KEY[1:"IF" TYPE=3 "THEN" NMAX "ELSE" 1],
 17            RNAMPOS[1:"IF" RNAMPRES "THEN" TMAX+1 "ELSE"1], ROWNAMES[1:"IF"
 18            RNAMPRES "THEN"5 *TMAX"ELSE"1], CNAMPOS[1:"IF" CNAMPRES"THEN"
 19            NMAX+1"ELSE"1], COLNAMES[1:"IF" CNAMPRES"THEN" 5* NMAX "ELSE"
 20            1], TBYN[1:TMAX,1:NMAX];
 21         "BEGIN" "COMMENT" SEGMENT;
 22            "COMMENT" P2IN5 IS A PROCEDURE STORED ON DISC TO READ A TBYN
 23               MATRIX FROM CARDS INTO AN INTEGER ARRAY AND TO CHECK FOR
 24               ERRORS IN THE DATA;


&CNLIST;


&LIST;
280            ERROR:="FALSE";




281            P2IN5 (TBYN, TMAX, T, NMAX, N, Q, TYPE, KEY, ROWNAMES, RNAMPOS,
282               RNAMPRES, COLNAMES, CNAMPOS, CNAMPRES);
283         "END" P2IN5 SEGMENT;
284         "BEGIN" "COMMENT" SEGMENT;
285            "INTEGER" S, STRIPS, PAGES, P, FIRSTB, LASTB, B, FIRSTC,
286               LASTC, C;
287            STRIPS:= (N-1) "DIV" 18 +1;
288            PAGES:=(T-1) "DIV" 50 + 1;
289            "FOR" S:=1 "STEP" 1 "UNTIL" STRIPS "DO"
290            "BEGIN" FIRSTC:=18*(S-1) + 1;
291               LASTC:=18*S;
292               "FOR" P:=1 "STEP" 1 "UNTIL" PAGES "DO"
293               "BEGIN" "PRINT" ''F'';
294                  FIRSTB:=50*(P-1)+1;
295                  LASTB:=50*P;
296                  "IF" LASTB>T "THEN" LASTB:=T;
297                  "FOR" B:=FIRSTB "STEP" 1 "UNTIL" LASTB "DO"
298                  "BEGIN" "PRINT" ''LS20'';
299                     "IF" LASTC>N "THEN" LASTC:=N;
300                     "FOR"C:=FIRSTC "STEP"1 "UNTIL" LASTC "DO"
301                        "PRINT" SAMELINE, DIGITS(2), TBYN[B,C]
302                  "END" B
303               "END" P
304            "END" S
305         "END" PRINTING SEGMENT
306      "END" DYNAMIC BLOCK
307   "END" PROGRAM;
```

(P2IN5 is a procedure used to read the t by n matrix from cards and to check for errors, and is used in several of the numerical taxonomy programs).

IV. Serological Methods.

Bacterial strains for antiserum production, were selected to represent the four main serological types of Listeria monocytogenes (Seeliger (1961)), L. grayi, L. murrayi, L. denitrificans and most of the main groups included in the numerical taxonomic survey. Antisera for the Streptococcus serological groups were obtained from the Wellcome Foundation (Langley Court, Beckenham, Kent). TABLE 10 contains a complete list of the strains included in the serological study. The streptococcal serological groups P, R and S were not represented in the numerical taxonomic survey, but were included here for completeness.

It was also thought worthwhile to examine serologically representatives of the three groups of Gram positive bacteria isolated by Thornley and Sharpe (1959) from chicken meat. Thornley and Sharpe reported that these bacteria resembled both lactobacilli and aerobic sporeformers. Jones (pers. comm.) in a numerical taxonomic study, demonstrated that a representative of group I was a member of the species Microbacterium thermosphactum, that a representative of group II closely resembled the lactobacilli, and that a member of the third group resembled Listeria. The representatives of these three groups will be refered to as T.S.I, T.S.II and T.S.III respectively (see TABLE 10). Antisera were not prepared against these bacteria, but HCl extracts

TABLE 10. List of Strains used in the Serological Survey.

Listeria monocytogenes	(serotype 1a)	G 32	
" "	(serotype 2)	G 22	
" "	(serotype 3a)	G 29	
" "	(serotype 4a)	G 30	
" grayi		G 42	
" murrayi		G 44	
" denitrificans		G 43	
" "		G 1	+
Lactobacillus bulgaricus		G 64	
" casei		G 65	
" delbrueckii		G 68	1
Gemella haemolysans		G 82	
Microbacterium thermosphactum		G104	
Erysipelothrix rhusiopathiae		G190	
Corynebacterium poinsettiae		G158	
Kurthia zopfii		G168	
Streptococcus pyogenes	group A	G120	
" agalactiae	" B	G121	
" equi	" C	G122	
" durans	" D	G124	
" faecalis	" D	G125	
" sp.	" E	G129	
" sp.	" F	G130	
" sp.	" G	G131	
" sp.	" H	G132	
" sp.	" K	G133T	
" sp.	" L	G 48	

TABLE 10. (continued).

Streptococcus	sp.	group	M	G135	
"	sp.	"	N	G136	
"	sp.	"	O	G137	
"	sp.	"	P	G 49, NCTC 9824	*
"	sp.	"	Q	G134	
"	sp.	"	R	G 50, NCTC 10234	*
"	sp.	"	S	G 51, NCTC 10237	*
T.S.I				LTRS MT77	+ *
T.S.II				LTRS MT 1	+ *
T.S.III				LTRS MT36	+ *

\+ no antisera produced, but HCl extracts were tested.

* not included in the numerical taxonomic survey.

1 only 'H' antigens tested.

NCTC National Collection of Type Cultures, Central Public Health Laboratories, Colindale Avenue, London.

LTRS Low Temperature Research Station, Cambridge. (collection now held at the Food Research Institute, Colney Lane, Norwich.).

were tested with antisera against the other bacteria.

Vaccines and HCl extracts were prepared as in section (a) below, and the vaccines were then used for antiserum production as in section (b). Slide and tube agglutination tests, the ring precipitin test and double gel diffusion technique (see section (c)) were used to study the various antisera.

All the early work and much of the present serological work with Listeria has involved agglutination tests for both somatic and flagella antigens. HCl extracts were first used by Lancefield (1933) working with the streptococci. She intimated in early papers that they were polysaccharide extracts, and later work by Elliott (1959) showed that teichoic acids were infact involved. Sharpe (1955) obtained good groupings for the lactobacilli with HCl extracts, and it appears that, within the whole of this lactic group, the HCl extract is a good method. It may well be that teichoic acids are involved throughout the group; Keeler and Gray (1960) investigated the cell walls of L. monocytogenes and found that they contained phosphorous, indicative of the presence of teichoic acids. There are reports (Seeliger (1961) and Gray and Killinger (1966)) that when ring precipitin or double gel diffusion tests, with HCl extracts, are used to study the somatic antigens of Listeria less equivocal results are obtained. Gray and Killinger (1966) also indicated that immunofluorescence techniques were most efficient for clinical material, the advantages for the detection of Listeria in infected tissues are obvious, particularly in view of the problems involved in its isolation (Mair (1968) and Kramer

and Jones (1969)). Obviously such techniques were beyond the scope of the present study, and for normal serology it appears that HCl extracts tested by the ring precipitin and double gel diffusion techniques are perfectly acceptable, if not preferable. For this reason, HCl extracts were used in the present study, but homologous titres were obtained by tube agglutination.

All somatic antisera were tested with homologous HCl extracts by the ring precipitin test to check that a positive reaction occurred by this technique. These antisera were then tested against HCl extracts of the selected strains, positive reactions were further investigated by double gel diffusion, and when possible, suitable absorptions were performed.

The flagella antisera were tested by tube agglutination with particulate antigen suspensions of the various strains to be tested (see TABLE 11). The antisera were then absorbed with homologous somatic antigens and the reactions with the various strains were retested, this time mainly by slide agglutination, but in some instances by tube agglutination.

a) Preparation of Antigens.

Preparation of Vaccines. The strains selected for vaccine production are listed in TABLE 12 . The majority of strains were grown in 500ml. quantities of Nutrient Broth No. 2 (Oxoid CM 67) plus 0.5%w/v glucose (NBG), in 500ml. screw cap bottles. For the lactobacilli, however, Sharpe's Medium A was used (Sharpe (1955)), and since K. zopfii and C. poinsettiae did not achieve sufficient growth in static cultures,

TABLE 11. Strains of which the flagella antigens were tested.

Listeria monocytogenes	(serotype 1a)	G32	*
" "	(serotype 2)	G22	*
" "	(serotype 3a)	G29	*
" "	(serotype 4a)	G30	*
" grayi		G42	*
" murrayi		G44	
" denitrificans		G43	
" "		G1	
Lactobacillus delbrueckii		G68	
Streptococcus pyogenese		G125	
Corynebacterium poinsettiae		G158	
Kurthia zopfii		G168	
one strain from groupIII of Thornley and Sharpe (1959)		T.S.III	

* Strains which were used for antiserum production.

TABLE 12. Strains used for vaccine production and their incubation conditions.

Strains	Survey number	Antigen type	Medium	Incubation-Temp.	Incubation-period	Static/Shaken
Listeria monocytogenes serotype 1a	G 32	'O'	NBG	35°C	24 hours	static
" " " 1a	G 32	'H'	"	5°C	3 weeks	"
" " " 2	G 22	'O'	"	35°C	24 hours	"
" " " 2	G 22	'H'	"	5°C	3 weeks	"
" " " 3a	G 29	'O'	"	35°C	24 hours	"
" " " 3a	G 29	'H'	"	5°C	3 weeks	"
" " " 4a	G 30	'O'	"	35°C	24 hours	"
" " " 4a	G 30	'H'	"	5°C	3 weeks	"
" grayi	G 42	'O'	"	35°C	24 hours	"
" "	G 42	'H'	"	5°C	3 weeks	"
" murrayi	G 44	'O'	"	35°C	24 hours	"
" denitrificans	G 43	'O'	"	"	"	"
Lactobacillus bulgaricus	G 64	'O'	Sharpe's A	"	"	"
" casei	G 65	'O'	"	"	"	"
Gemella haemolysans	G 82	'O'	NBG	"	3 days	"

TABLE 12. (continued).

Strains	Survey number	Antigen type	Medium	Incubation Temp.	Incubation Period	Static/ Shaken
Microbacterium thermosphactum	G 104	'O'	NBG	30°C	3 days	static
Erysipelothrix rhusiopathiae	G 190	'O'	"	35°C	3 days	"
Corynebacterium poinsettiae	G 158	'O'	"	30°C	2 days	shaken
Kurthia zopfii	G 168	'O'	"	"	"	"

NBG Nutrient Broth No. 2 (Oxoid CM 67) plus 0.05%w/v glucose.

Sharpe's A Medium A of Sharpe (1955).

'O' somatic antigen.

'H' flagella antigen.

they were shaken in 500ml. quantities of NBG in 2l. flasks. The Listeria cultures, to be used for the production of flagella 'H' antibodies, were grown at 4°C since a lower temperature of incubation was reported to enhance flagellation (Larsen and Seeliger (1966)). Full details of growth conditions and periods of incubation, for both somatic 'O' and flagella 'H' antigens, are given in TABLE 12 . When good growth had been achieved, two drops from each of the broths were plated onto BM agar of Blood agar (Oxoid CM 55), to test for purity, and 0.04% formaldehyde was added to those broths to be used for the preparation of 'H' antigens. The growth was sedimented by centrifugation at 3000g. for 20min. using an M.S.E. high speed 18 centrifuge, and resuspended in 50 ml. of sterile physiological saline. This process was repeated twice, the final bacterial sediment was resuspended in 10 to 20ml. of sterile physiological saline and placed in a sterile bottle.

The 'H' antigen suspensions were preserved by the addition of 0.04% formaldehyde. The 'O' antigen suspensions were heated in a boiling water-bath for 1 hour, and on cooling 5 drops of a 1%w/v merthiolate solution were added as a preservative. All vaccines were stored at 4°C and, before use, were tested for sterility and adjusted to Brown's opacity tube (BT) numbers 4 and 8. Suspensions at BT 4 were used for initial injections, but subsequently suspensions at BT 8 were employed.

Preparation of Particulate Antigens. (As for the preparation of vaccines).

Preparation of the HCl Extracts. The method of Lancefield (1933) was used. The bacteria were grown in 100ml. quantities of Glucose-Lemco Broth in 100ml. screw cap bottles at 37°C, until good growth was achieved (usually 1-2 days). K. zopfii and C. poinsettiae again required aeration. M. thermosphactum was grown at 30°C, and this bacterium, E. rhusiopathiae and G. haemolysans were cultured in NBG to achieve sufficient growth. All the bacteria were sedimented by centrifugation at 3,000g. for 20min. in an M.S.E. high speed 18 centrifuge. The bacterial pellet was resuspended in 2ml. of sterile 0.05% hydrochloric acid in 0.85% sodium chloride solution. The resultant suspension was heated for 10min. in a boiling water-bath, cooled immediately in cold water and neutralized with sterile 0.5N sodium hydroxide solution, using one drop of phenol red as indicator. The cell debris was then sedimented by centrifugation for 20min. in an M.S.E. bench centrifuge. The supernatant was carefully removed to a sterile bijoux bottle. Two drops of a 1%w/v merthiolate solution were added as a preservative. The HCl extracts were stored at 4°C.

b) Preparation of Antisera.

Sandy-Lop Rabbits (Norfolk Rabbits, Bun's Bank, Norwich) were injected intravenously in the left ear, with 0.5ml to 2ml. of the sterile antigen preparations. Initially, a course of six injections, spaced over two weeks, was given. Test bleedings were made 7 to 10 days after the last injection. The blood was 'ringed', allowed to clot at 37°C for 1 hour before the serum was removed. Any blood cells were

sedimented by centrifugation for 20min. in an M.S.E. bench centrifuge. The titre was determined by tube agglutination tests (see section (d)), and if a reaction was observed at a serum dilution of 1 in 512 or over a full bleed was performed the following day. Sera were harvested as described above, and both test and full bleed antisera were preserved by the addition of one drop of a 1%w/v merthiolate solution per 3ml. and stored at 4°C.

Absorption. The strains required for absorption were grown in 250ml. quantities of NBG in 250ml. screw cap bottles, at 35°C. The growth was sedimented by centrifugation in an M.S.E. high speed 18 centrifuge at 3,000g. for 20 min. The pellet was resuspended in 5 to 10 ml. of sterile physiological saline, heated in a boiling water bath for 1 hour and sedimented by centrifugation in an M.S.E. bench centrifuge for 20min. The antiserum to be absorbed was poured onto the resultant pellet and incubated at 35°C. At intervals the cells were sedimented using an M.S.E. bench centrifuge, and the antiserum tested by slide agglutination with somatic antigen of the organism being used for absorption. When agglutination could no longer be detected by slide agglutination, the absorption was considered to be complete and the antiserum removed from the cell pellet.

c) Serological Test Methods.

Tube Agglutination Test. The antiserum to be tested was diluted by a series of doubling dilutions in 0.5ml. amounts of physiological saline (from 1 in 2 to 1 in 1024), in agglutination tubes. Particulate antigen at BT 4 was diluted

by adding 2ml. of antigen to 5ml. of physiological saline. The resultant antigen suspension (0.5ml.) was added to each of the serum dilutions and a saline control (to detect any autoagglutination). The antigen and antiserum were mixed well, and incubated in a water-bath at 37°C overnight (approximately 18 hours), or in a water-bath at 50°C for 1 hour followed by overnight incubation at 4°C. The tubes were then observed for agglutination (clumping of the antigen suspension).

Slide Agglutination Test. One drop of the antiserum to be tested and one drop of saline (as a control to test for autoagglutination) were placed on a clean glass slide. One drop of antigen suspension was then added to each of these drops, the slide rocked to ensure good mixing, and the drops observed for agglutination.

Ring Precipitin Test. Small quantities of antiserum were placed at the bottom of 2mm. diameter glass tubes. Similar amounts of the HCl extracts were layered carefully upon the antiserum, and to one saline was added as a control. The tubes were left to stand at room temperature, and observations were made after 10min., 30min. and 1 hour for the production of a white ring at the interface of the two liquids, indicative of an interaction between the antigen and the antibody.

Double Gel Diffusion Test. The method was based upon the Mansi(1958) modification of the double gel diffusion method of Ouchterlony (1953). Five ml. of Ouchterlony agar were carefully layered onto a 1" by 3" glass slide. When set, wells were cut into the agar such that six wells were equi-

distant from a central seventh well. A metal template was used for this purpose. The antiserum to be tested was placed in the central well, and the antigens (HCl extracts) and a saline control were placed in the outer wells. The slides were incubated at room temperature in a humidity chamber, and observations were made after 1, 2 and 3 days. A reaction was indicated by the development of a line of whitish precipitate in the agar between the wells.

RESULTS.

RESULTS.

I. Numerical Taxonomy.

The coded results (see Appendix IV) were analysed as described in the Computer Methods section and the relevant dendrograms and similarity matrices constructed. The dendrograms are represented in Figures 2,3,4 and 6, and the similarity matrices are shown in Figure 5 and Appendix III Figures I to IV.

The large size of the data matrix and limitations imposed by the computer did not permit all of the OTUs to be compared at the same time. For a data matrix in which there were 143 characters a maximum of 180 OTUs could be compared, therefore, 12 of the total of 192 OTUs included in the survey were omitted. The 25 strains of M. thermosphactum were very similar, and it was, therefore, decided to delete 12 of these for the initial computation. (Subsequent computer analysis showed that the 25 strains of M. thermosphactum were at least 80% similar to each other, the majority more than 90% similar. See Appendix III Figure III).

The average (UPGM) and single (SLM) linkage clustering options were employed, but the former resulted in the production of a more informative dendrogram. As a result of the clustering method, the linkages between the main clusters in the SLM dendrogram tended to be less clear than those in the UPGM dendrogram. Using the SLM an OTU or a cluster was joined to an existing cluster at the similarity level it showed to the most similar OTU within that cluster. With the UPGM, however, the similarity level shown was the average

similarity between the entering OTU or cluster and the existing cluster (Sneath (1966)). Clustering, therefore, occurred at a higher similarity level with the SLM than with the UPGM, with the result that the linkages were compressed into a narrower similarity range.

The UPGM dendrogram produced for 180 OTUs (Dendrogram A) is shown in Figure 2 and the corresponding similarity matrix is in Appendix III Figure I .

Due to the necessity for two incubation temperatures within the survey (see Methods section I (b)), 46 strains were tested at 30°C and 35°C. These duplicate sets of results, plus the single sets of results for a further 88 strains, were compared using the program ITBNCLST. The dendrogram (Dendrogram B, Figure 3) and similarity matrix (Similarity Matrix B, Appendix III, Figure II) produced, indicated that there was in fact good agreement between the two sets of data, and that comparison of bacteria tested at different temperatures was, in this instance, acceptable.

In Dendrogram A (Figure 2) at approximately 67% similarity (S), the bacteria under study fell into two main clusters, labelled I and II.

Cluster I contained representatives of the genera Erysipelothrix, Gemella, Lactobacillus, Listeria, Propionibacterium and Streptococcus, together with the species Microbacterium thermosphactum, the so-called Bacterium eurydice, and one representative of the genus Brevibacterium - Brevibacterium divaricatum.

Cluster II contained representatives of various genera of the families Corynebacteriaceae, Brevibacteriaceae and

Figure 2. Dendrogram A.

(180 OTUs)

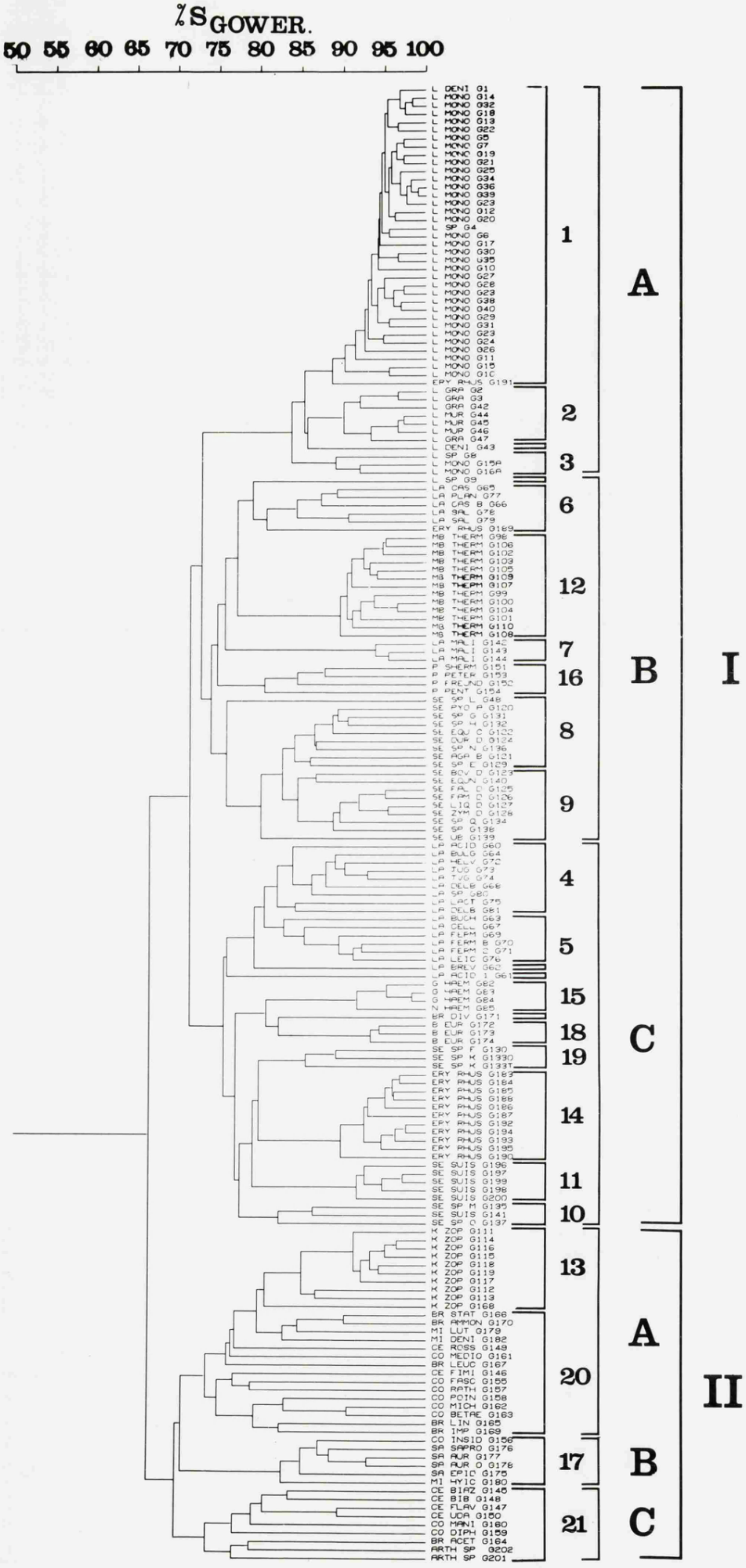
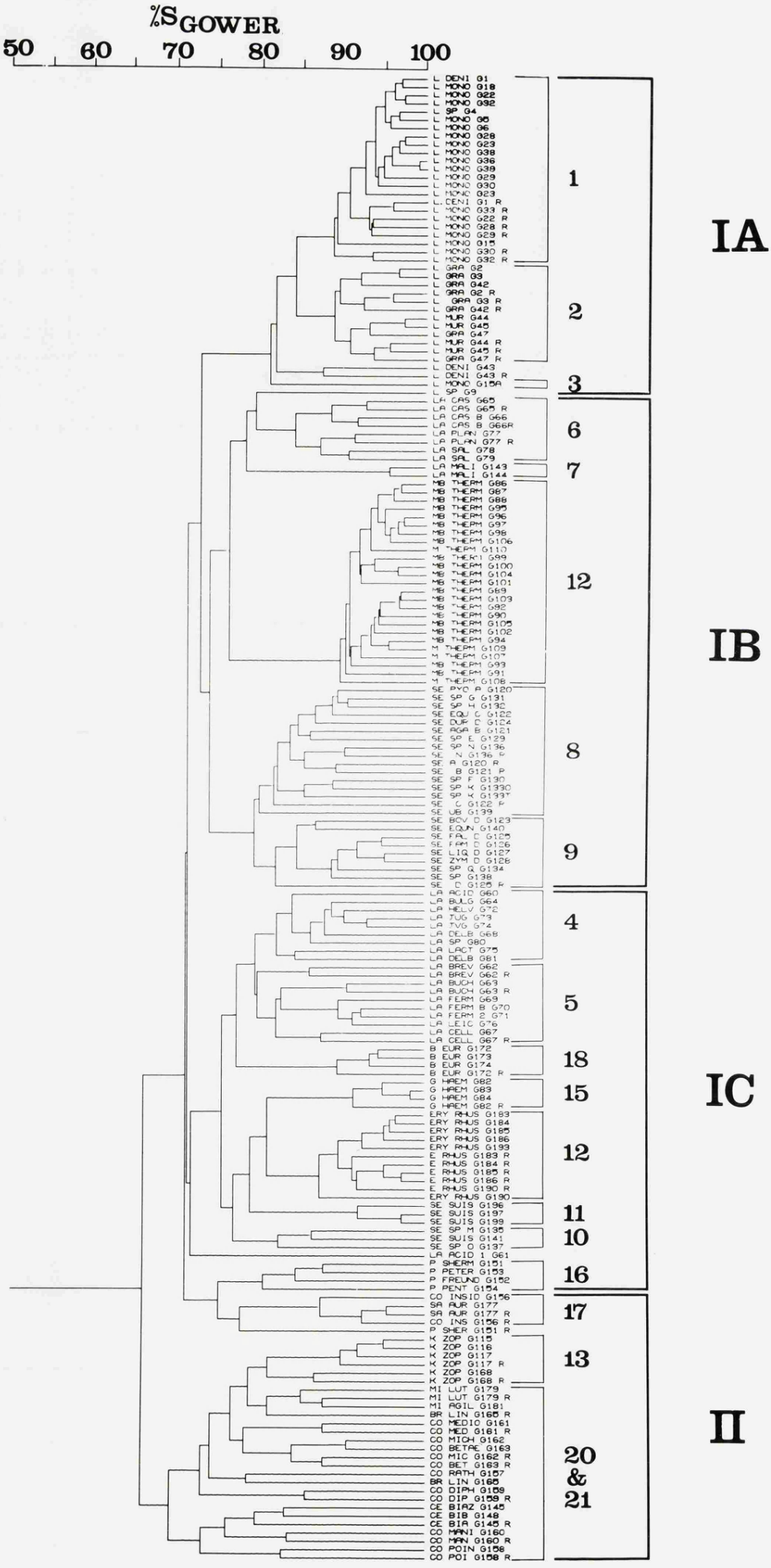

%S GOWER.
50 55 60 65 70 75 80 85 90 95 100
L DEN1 G1
L MONO G14
L MONO G32
L MONO G18
L MONO G13
L MONO G22
L MONO G5
L MONO G7
L MONO G19
L MONO G21
L MONO G25
L MONO G34
L MONO G36
L MONO G39
L MONO G23
L MONO G12
L MONO G20
L SP G4
L MONO G6
L MONO G17
L MONO G30
L MONO G35
L MONO G10
L MONO G27
L MONO G28
L MONO G33
L MONO G38
L MONO G40
L MONO G29
L MONO G31
L MONO G23
L MONO G24
L MONO G26
L MONO G11
L MONO G15
L MONO G1C
ERY RHUS G191
L GRA G2
L GRA G3
L GRA G42
L MUR G44
L MUR G45
L MUR G46
L GRA G47
L DEN1 G43
L SP G8
L MONO G15A
L MONO G16A
L SP G9
LA CAS G65
LA PLAN G77
LA CAS B G66
LA BAL G78
LA SAL G79
ERY RHUS G189
MB THERM G98
MB THERM G106
MB THERM G102
MB THERM G103
MB THERM G105
MB THERM G109
MB THERM G107
MB THERM G99
MB THERM G100
MB THERM G104
ME THERM G101
MB THERM G110
MB THERM G108
LA MALI G142
LA MALI G143
LA MALI G144
P SHERM G151
P PETER G153
P FREUND G152
P PENT G154
SE SP L G48
SE PYO P G120
SE SP G G131
SE SP H G132
SE EQU C G122
SE DUR D G124
SE SP N G136
SE AGA B G121
SE SP E G129
SE BOV D G123
SE EQU G140
SE FAL D G125
SE FPM D G126
SE LIQ D G127
SE ZYM D G128
SE SP Q G134
SE SP G138
SE UE G139
LA ACID G60
LA BULG G64
LA HELV G72
LA IUG G73
LA TUR G74
LA DELB G66
LA SP G80
LA LACT G75
LA DELB G61
LA BUCH G63
LA CELL G67
LA FERM G69
LA FERM B G70
LA FERM C G71
LA LEIC G76
LA BREV G62
LA ACID 1 G68
G NAEM G82
G NAEM G83
G NAEM G84
N NAEM G85
BR DIV G171
B EUR G172
B EUR G173
B EUR G174
SE SP F G130
SE SP K G1330
SE SP K G133T
ERY RHUS G183
ERY RHUS G184
ERY RHUS G185
ERY RHUS G188
ERY RHUS G186
ERY RHUS G187
ERY RHUS G192
ERY RHUS G194
ERY RHUS G193
ERY RHUS G195
ERY RHUS G190
SE SUIS G196
SE SUIS G197
SE SUIS G199
SE SUIS G198
SE SUIS G200
SE SP M G135
SE SUIS G141
SE SP O G137
K ZOP G111
K ZOP G114
K ZOP G116
K ZOP G115
K ZOP G118
K ZOP G119
K ZOP G117
K ZOP G112
K ZOP G113
K ZOP G168
ER STAT G166
BR AMMON G170
MI LUT G179
MI DEN1 G182
CE ACOS G149
CO MEDIO G161
BR LEUC G167
CE FIMI G146
CO FASC G155
CO RATH G157
CO POIN G158
CO MICH G162
CO BETAE G163
BR LIN G165
BR IMP G169
CO INSID G156
SA SAPRO G176
SA AUR G177
SA AUR O G178
SA EPID G175
MI HYIC G180
CE BIAZ G145
CE BIB G148
CE FLAV G147
CE UDA G150
CO MANI G160
CO DIPH G159
BR ACET G164
ARTH SP G202
ARTH SP G201
1
2
3
6
12
7
16
8
9
4
5
15
18
19
14
11
10
13
20
17
21
A
B
C
I
A
B
C
II

Figure 3. Dendrogram B.
(180 OTUs)


%S GOWER
50
60
70
80
90
100
L DENI G1
L MONO G18
L MONO G22
L MONO G32
L SP G4
L MONO G6
L MONO G26
L MONO G28
L MONO G23
L MONO G38
L MONO G36
L MONO G39
L MONO G29
L MONO G30
L MONO G23
L DENI G1 R
L MONO G33 R
L MONO G22 R
L MONO G28 R
L MONO G29 R
L MONO G15
L MONO G30 R
L MONO G32 R
L GRA G2
L GRA G3
L GRA G42
L GRA G2 R
L GRA G3 R
L GRA G42 R
L MUR G44
L MUR G45
L GRA G47
L MUR G44 R
L MUR G45 R
L GRA G47 R
L DENI G43
L DENI G43 R
L MONO G15A
L SP G9
LA CAS G65
LA CAS G65 R
LA CAS B G66
LA CAS B G66R
LA PLAN G77
LA PLAN G77 R
LA SAL G78
LA SAL G79
LA MALI G143
LA MALI G144
MB THERM G86
MB THERM G87
MB THERM G88
MB THERM G95
MB THERM G96
MB THERM G97
MB THERM G98
MB THERM G106
M THERM G110
MB THERM G99
MB THERM G100
MB THERM G104
MB THERM G101
MB THERM G89
MB THERM G103
MB THERM G92
MB THERM G90
MB THERM G105
MB THERM G102
MB THERM G94
M THERM G109
M THERM G107
MB THERM G93
MB THERM G91
M THERM G108
SE PYO A G120
SE SP G G131
SE SP H G132
SE EQU C G122
SE DUR D G124
SE AGA B G121
SE SP E G129
SE SP N G136
SE N G136 R
SE A G120 R
SE B G121 R
SE SP F G130
SE SP K G133O
SE SP K G133T
SE C G122 R
SE UB G139
SE BOV D G123
SE EQUN G140
SE FAL D G125
SE FAM D G126
SE LIQ D G127
SE ZYM D G128
SE SP Q G134
SE SP G138
SE D G125 R
LA ACID G60
LA BULG G64
LA HELV G72
LA JUG G73
LA TVG G74
LA DELB G68
LA SP G80
LA LACT G75
LA DELB G81
LA BREV G62
LA BREV G62 R
LA BUCH G69
LA BUCH G63 R
LA FERM G69
LA FERM B G70
LA FERM 2 G71
LA LEIC G76
LA CELL G67
LA CELL G67 R
B EUR G172
B EUR G173
B EUR G174
B EUR G172 R
G HAEM G82
G HAEM G83
G HAEM G84
G HAEM G82 R
ERY RHUS G183
ERY RHUS G184
ERY RHUS G185
ERY RHUS G186
ERY RHUS G199
E RHUS G183 R
E RHUS G184 R
E RHUS G185 R
E RHUS G186 R
E RHUS G190 R
ERY RHUS G190
SE SUIS G196
SE SUIS G197
SE SUIS G198
SE SP M G135
SE SUIS G141
SE SP O G137
LA ACID 1 G61
P SHERM G151
P PETER G153
P FREUND G152
P PENT G154
CO INSID G156
SA AUR G177
SA AUR G177 R
CO INS G156 R
P SHER G151 R
K ZOP G115
K ZOP G116
K ZOP G117
K ZOP G117 R
K ZOP G168
K ZOP G168 R
MI LUT G179
MI LUT G179 R
MI AGIL G181
BR LIN G165 R
CO MEDIO G161
CO MED G161 R
CO MICH G162
CO BETAE G163
CO MIC G162 R
CO BET G163 R
CO RATH G157
BR LIN G165
CO DIPH G159
CO DIP G159 R
CE BIAZ G145
CE BIB G148
CE BIA G145 R
CO MANI G160
CO MAN G160 R
CO POIN G158
CO POI G158 R
1
2
3
6
7
12
8
9
4
5
18
15
12
11
10
16
17
13
20
&
21
IA
IB
IC
II

Micrococcaceae, which were included for comparitive purposes.

These two main clusters divided into a total of 21 minor clusters, the membership of which is shown in TABLE 13.

Cluster I.

Within this cluster three main subdivisions, IA, IB and IC, were formed above the 75% similarity level (S).

Cluster IA.

This cluster was a relatively homogeneous group with an overall similarity of about 80% S and contained all but one of the strains received as Listeria species, plus one strain received as Erysipelothrix rhusiopathiae. Three clusters and one single strain (L. denitrificans) could be distinguished within the cluster. The first cluster, IA1, (88%S) contained 35 strains received as L. monocytogenes and Listeria sp., one strain received as E. rhusiopathiae and one strain received as L. denitrificans (G1). The second cluster, IA2, (90%S) contained all the strains of L. grayi and L. murrayi, and the final cluster, IA3, (82%S) contained three strains (G8, G15a and G16a) of L. monocytogenes and Listeria sp. Strain G8, which was non-haemolytic, was received from the collection of the late E.G.D. Murray as a possible representative of a new species of Listeria. Strains G15 and G16, Listeria monocytogenes isolated from salmon, contained two types of colony on receipt, one translucent and β-haemolytic (typical of L. monocytogenes), the other more opaque and non-haemolytic. The two types were separated and the less typical forms referred to as G15a and G16a.

TABLE 13. The composition of the clusters formed in Dendrogram A. (Figure 2).

Cluster IA1.

Listeria denitrificans G1.

Listeria monocytogenes G5, G6, G7, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24, G25, G26, G27, G28, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40.

Listeria sp. G4.

Erysipelothrix rhusiopathiae G191.

Cluster IA2.

Listeria grayi G2, G3, G42, G47.

Listeria murrayi G44, G45, G46.

Cluster IA3.

Listeria monocytogenes G15a, G16a.

Listeria sp. G8.

Cluster IB6.

Lactobacillus casei G65, G66.

" *plantarum* G77.

" *salivarius* G78, G79.

Erysipelothrix rhusiopathiae G189.

Cluster IB7.

Lactobacillus mali G142, G143, G144.

TABLE 13. (continued).

Cluster IB8.

Streptococcus pyogenes G120.
" agalactiae G121.
" equi H122.
" durans G124.
" sp. (E) G129.
" sp. (G) G131.
" sp. (H) G132.
" sp. (N) G136.

Cluster IB9.

Streptococcus bovis G123.
" faecalis G125.
" faecium G126.
" liquifaciens G127.
" zymogenes G128.
" sp. (Q) G134.
" sp. G138.
" equinus G140.

Cluster IB12.

Microbacterium thermosphactum G98, G99, G100, G101, G102, G103, G104, G105, G106, G107, G108, G109, G110.

Cluster IB16.

Propionbacterium shermanii G151.
" freundenreichii G152.

TABLE 13. (continued).

Cluster IB16 cont.

Propionibacterium petersonii G153.

" *pentosaceum* G154.

Cluster IC4.

Lactobacillus acidophilus G60.

" *bulgaricus* G64.

" *delbrueckii* G68, G81.

" *helveticus* G72.

" *jugurt* G73, G74.

" *lactis* G75.

" sp. G80.

Cluster IC5.

Lactobacillus buchneri G63.

" *cellobiosus* G67.

" *fermentum* G69, G70, G71.

" *leichmannii* G76.

Cluster IC10.

Streptococcus sp. (M) G135.

" sp. (O) G137.

" *suis* G141.

Cluster IC11.

Streptococcus suis G196, G197, G198, G199, G200.

TABLE 13. (continued).

Cluster IC14.

Erysipelothrix rhusiopathiae G183, G184, G185, G186, G187, G188, G190, G192, G193, G194, G195.

Cluster IC15.

Gemella haemolysans G82, G83, G84.

Neisseria haemolysans G85.

Cluster IC18.

Bacterium eurydice G172, G173, G174.

Cluster IC19.

Streptococcus sp. (F) G130.

" sp. (K) G1330, G133T.

Cluster IIA13.

Kurthia zopfii G111, G112, G113, G114, G115, G116, G117, G118, G119, G168.

Cluster IIA20.

Brevibacterium linens G165.

" stationis G166.

" leucinophagum G167.

" imperiale G169.

" ammoniagenes G170.

Cellulomonas fimi G146.

" rossica G149.

TABLE 13. (continued).

Cluster IIA20 cont.

Corynebacterium fascians G155.
" *rathayi* G157.
" *poinsettiae* G158.
" *mediolanum* G161.
" *michiganense* G162.
" *betae* G163.
Micrococcus luteus G179.
" *denitrificans* G182.

Cluster IIB17.

Corynebacterium insidiosum G156.
Micrococcus hyicus G180.
Staphylococcus epidermidis G175.
" *saprophyticus* G176.
" *aureus* G177, G178.

Cluster IIC21.

Arthrobacter sp. G201, G202.
Brevibacterium acetylicum G164.
Cellulomonas biazotea G145.
" *flavigena* G147.
" *bibula* G148.
" *uda* G150
Corynebacterium diphtheriae G159.
" *manihot* G160.

Cluster IB.

This relatively heterogeneous group with much internal structure, contained all strains received as Lactobacillus mali, M. thermosphactum and Propionibacterium, most of the strains of Streptococcus, 5 strains of the genus Lactobacillus, one strain of E. rhusiopathiae and one strain of Listeria sp. Above 80% S 6 clear clusters and 3 single strains could be distinguished within group IB. The three single strains were received as Listeria sp., Streptococcus sp. (serological group L) and S. uberis.

The 5 strains of Lactobacillus sp. and the one strain received as E. rhusiopathiae formed a cluster, IB6, (82% S) to which the single strain of Listeria sp. (G9) joined at 79% S. The strains of M. thermosphactum formed a tight cluster, IB12, (90% S) which joined cluster IB6 at 77% S. The 3 strains of L. mali, cluster IB7, (94% S) joined clusters IB6 and IB12 at 76% S, and the 4 strains of Propionibacterium sp., cluster IB16, (81% S) joined these three clusters at 75% S. The remaining strains within group IB were all streptococci. The streptococci of serological group D (except S. durans), plus one representative of group Q, formed a cluster, IB9, (85% S). All but two of the remaining streptococci within cluster IB (including S. durans) formed a cluster, IB8, at 84% S, and joined cluster IB9 at 82% S. These two clusters of streptococci were joined at 80% S by the single strain of S. uberis, and at 76% S by the Streptococcus strain G48 (serological group L). The streptococci then joined the remaining members of group IB (clusters IB6, IB7, IB12 and IB 16) at 74% S, and the clusters

IA and IB were linked at 73% S.

Cluster IC.

This cluster was also relatively heterogeneous. It contained 3 strains received as Bacterium eurydice, the single strain of Brevibacterium divaricatum, all but two strains of E. rhusiopathiae (see clusters IA and IB), members of the genus Gemella, the majority of the lactobacilli and the remaining strains of streptococci. Above 80% S, 8 clusters and 3 single strains (Lactobacillus brevis, one of the 2 strains of L. acidophilus and B. divaricatum) could be distinguished. The lactobacilli formed two main clusters, IC4 (82% S) and IC5 (83% S), to which L. brevis joined at 80% S, and L. acidophilus (biotype I) at 76% S. The strains of E. rhusiopathiae formed a very tight cluster, IC14, (90% S) which at 80%S was joined by a small cluster, IC19, (85% S) which contained three strains of streptococci from serological groups F and K. Five strains received as S. suis formed a tight cluster, IC11, (92% S), which joined clusters IC14 and IC19 at 79% S. Three further strains of streptococci, one received as S. suis, and one from each of the serological groups O and M, formed a cluster, IC10, (82% S) which joined the clusters IC11, IC14 and IC19 at 78% S. The 3 strains of B. eurydice formed a tight cluster, IC18, (94% S) to which the single strain of Brevibacterium divaricatum was joined at 82% S. The 4 strains of G. haemolysans formed a tight cluster, IC15, (92% S) which joined cluster IC18 and the strain of B. divaricatum at 81% S. These strains then joined clusters IC10, IC11, IC14 and IC19 at 77% S, and these 6 clusters then joined the two Lactobacillus clusters, IC4 and IC5, at 76% S.

The entire cluster, IC, then joined clusters IA and IB at 71% S.

Cluster II.

Within this cluster three main subdivisions, IIA, IIB and IIC could be distinguished above 72% S.

Cluster IIA.

This heterogeneous group possessed much internal structure, and contained 5 strains of the genus Brevibacterium, 6 from the genus Corynebacterium, two strains of Cellulomonas, 2 micrococci and all the strains received as Kurthia zopfii. At 81% S the strains of K. zopfii formed a relatively close cluster, IIA13. The remaining strains within cluster IIA, IIA20, formed a very heterogeneous group, three strains joined cluster IIA13 singly, the remainder did so in small, very loose clusters, and showed between 73 and 77% S to cluster IIA13.

Cluster IIB.

This cluster was a more homogeneous group. It contained 1 strain received as Corynebacterium insidiosum, 2 strains of Staphylococcus aureus, 1 strain of S. epidermidis, and 2 strains received as S. saprophyticus and Micrococcus hyicus. The latter two species are probably synonymous with S. epidermidis (Jones, Deibel and Niven (1963)). The cluster was relatively compact (83% S) and no clear subdivisions could be distinguished. It was, therefore, labelled as one cluster, IIB17, and it joined cluster IIA at 71% S.

Cluster IIC.

This was a very heterogeneous group, comprising 2 strains

received as possible members of the genus Arthrobacter, 1 strain of Brevibacterium acetylicum, 4 strains of the genus Cellulomonas, and 2 strains from the genus Corynebacterium. The cluster was very loosely formed, the majority of the strains being between 73 and 81% similar. No clear subdivisions were distinguishable and the entire group was, therefore, labelled as one cluster, IIC21, which joined clusters IIA and IIB at 70% S.

It was decided to investigate 137 of the strains included in Dendrogram A (Figure 2), plus the 12 strains of M. thermosphactum which had been omitted, in further detail. Using the program ITBNCLST, Dendrogram C (Figure 4) and Similarity Matrix C (Figure 5 and Appendix III, Figure III) were produced. The clusters formed were identical to, or closely resembled, those in Figure 2, and the main divisions into clusters IA, IB, IC and II could clearly be seen. The M. thermosphactum cluster, IB12, again formed at 90% S, but also contained the 12 additional strains of that species. Of the three strains which formed cluster IC19, in Figure 2, two (G130 and G1330) now fell in cluster IB8, while the other (G133T) remained as a single strain joining cluster IC5 at 75% S. The other clusters were as in Figure 2, see TABLE 14 for the group memberships. The similarity matrix from magnetic tape, plus details of the group memberships (for this purpose G133T, due to the similarity it showed to G130 and G1330 in Figure 2, was also included in cluster IB8) were used with the program IGROUPS to produce the TABLE I as shown in Appendix III Details of the number of '0', '1' and '2' results for each group, for each character, are shown in this TABLE, with the aid of which, it

Figure 4. Dendrogram C.

(149 OTUs)

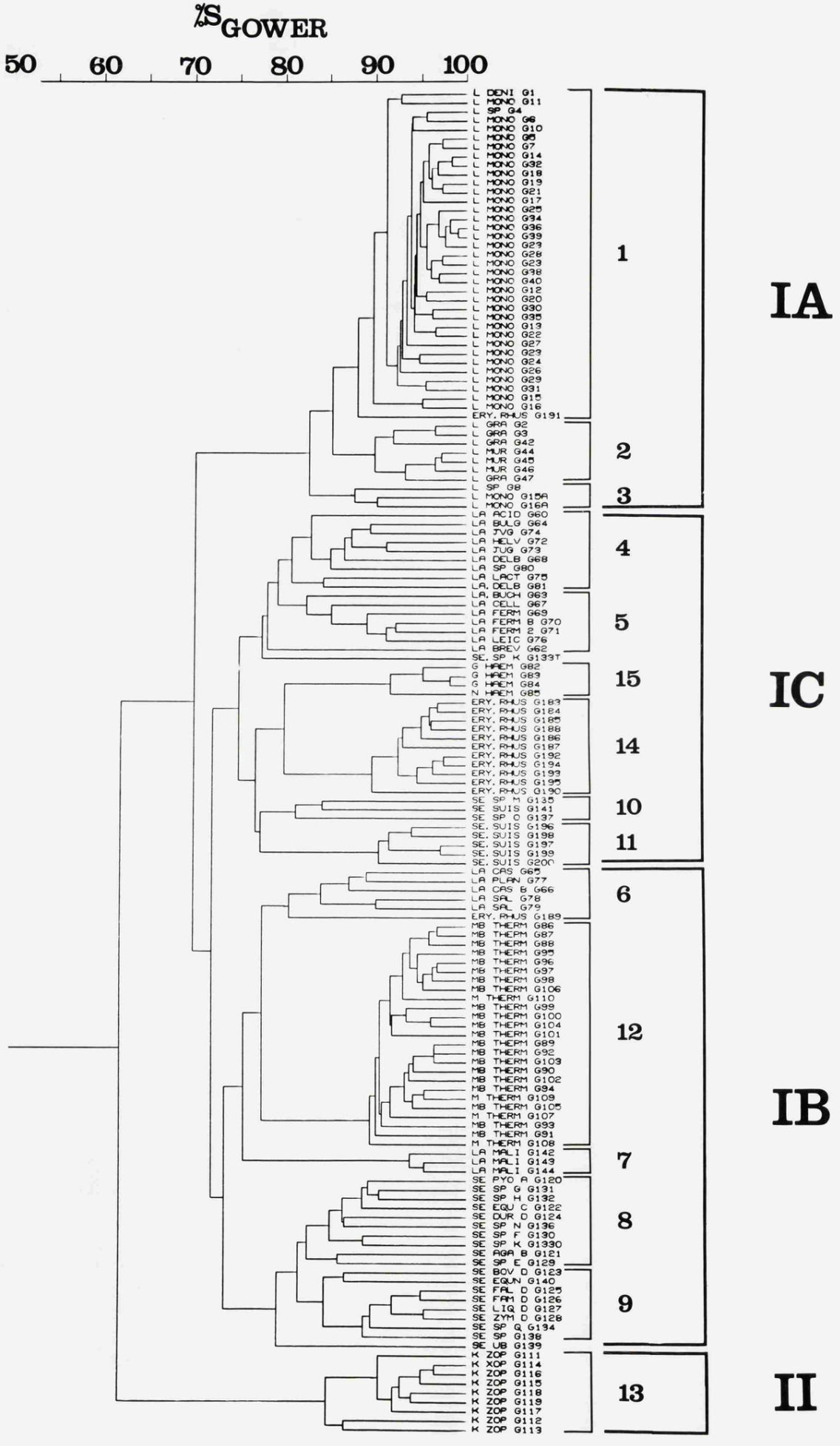

%S GOWER
50
60
70
80
90
100
L DENI G1
L MONO G11
L SP G4
L MONO G6
L MONO G10
L MONO G5
L MONO G7
L MONO G14
L MONO G32
L MONO G18
L MONO G19
L MONO G21
L MONO G17
L MONO G25
L MONO G34
L MONO G36
L MONO G39
L MONO G29
L MONO G28
L MONO G23
L MONO G38
L MONO G40
L MONO G12
L MONO G20
L MONO G30
L MONO G35
L MONO G13
L MONO G22
L MONO G27
L MONO G33
L MONO G24
L MONO G26
L MONO G29
L MONO G31
L MONO G15
L MONO G16
ERY. RHUS G191
L GRA G2
L GRA G3
L GRA G42
L MUR G44
L MUR G45
L MUR G46
L GRA G47
L SP G8
L MONO G15A
L MONO G16A
LA ACID G60
LA BULG G64
LA JUG G74
LA HELV G72
LA JUG G73
LA DELB G68
LA SP G80
LA LACT G75
LA. DELB G81
LA. BUCH G63
LA CELL G67
LA FERM G69
LA FERM B G70
LA FERM 2 G71
LA LEIC G76
LA BREV G62
SE. SP K G133T
G HAEM G82
G HAEM G83
G HAEM G84
N HAEM G85
ERY. RHUS G183
ERY. RHUS G184
ERY. RHUS G185
ERY. RHUS G188
ERY. RHUS G186
ERY. RHUS G187
ERY. RHUS G192
ERY. RHUS G194
ERY. RHUS G193
ERY. RHUS G195
ERY. RHUS G190
SE SP M G135
SE SUIS G141
SE SP O G137
SE. SUIS G196
SE. SUIS G198
SE. SUIS G197
SE. SUIS G199
SE. SUIS G200
LA CAS G65
LA PLAN G77
LA CAS B G66
LA SAL G78
LA SAL G79
ERY. RHUS G189
MB THERM G86
MB THERM G87
MB THERM G88
MB THERM G95
MB THERM G96
MB THERM G97
MB THERM G98
MB THERM G106
M THERM G110
MB THERM G99
MB THERM G100
MB THERM G104
MB THERM G101
MB THERM G89
MB THERM G92
MB THERM G103
MB THERM G90
MB THERM G102
MB THERM G94
M THERM G109
MB THERM G105
M THERM G107
MB THERM G93
MB THERM G91
M THERM G108
LA MALI G142
LA MALI G143
LA MALI G144
SE PYO A G120
SE SP G G131
SE SP H G132
SE EQU C G122
SE DUR D G124
SE SP N G136
SE SP F G130
SE SP K G133D
SE AGA B G121
SE SP E G129
SE BOV D G123
SE EQUN G140
SE FAL D G125
SE FAM D G126
SE LIQ D G127
SE ZYM D G128
SE SP Q G134
SE SP G138
SE UB G139
K ZOP G111
K XOP G114
K ZOP G116
K ZOP G115
K ZOP G118
K ZOP G119
K ZOP G117
K ZOP G112
K ZOP G113
1
2
3
4
5
15
14
10
11
6
12
7
8
9
13
IA
IC
IB
II

Figure 5. Similarity Matrix C.

(149 OTUs)

Key to Similarity Levels:

90% S

80% S

70% S

60% S

50% S

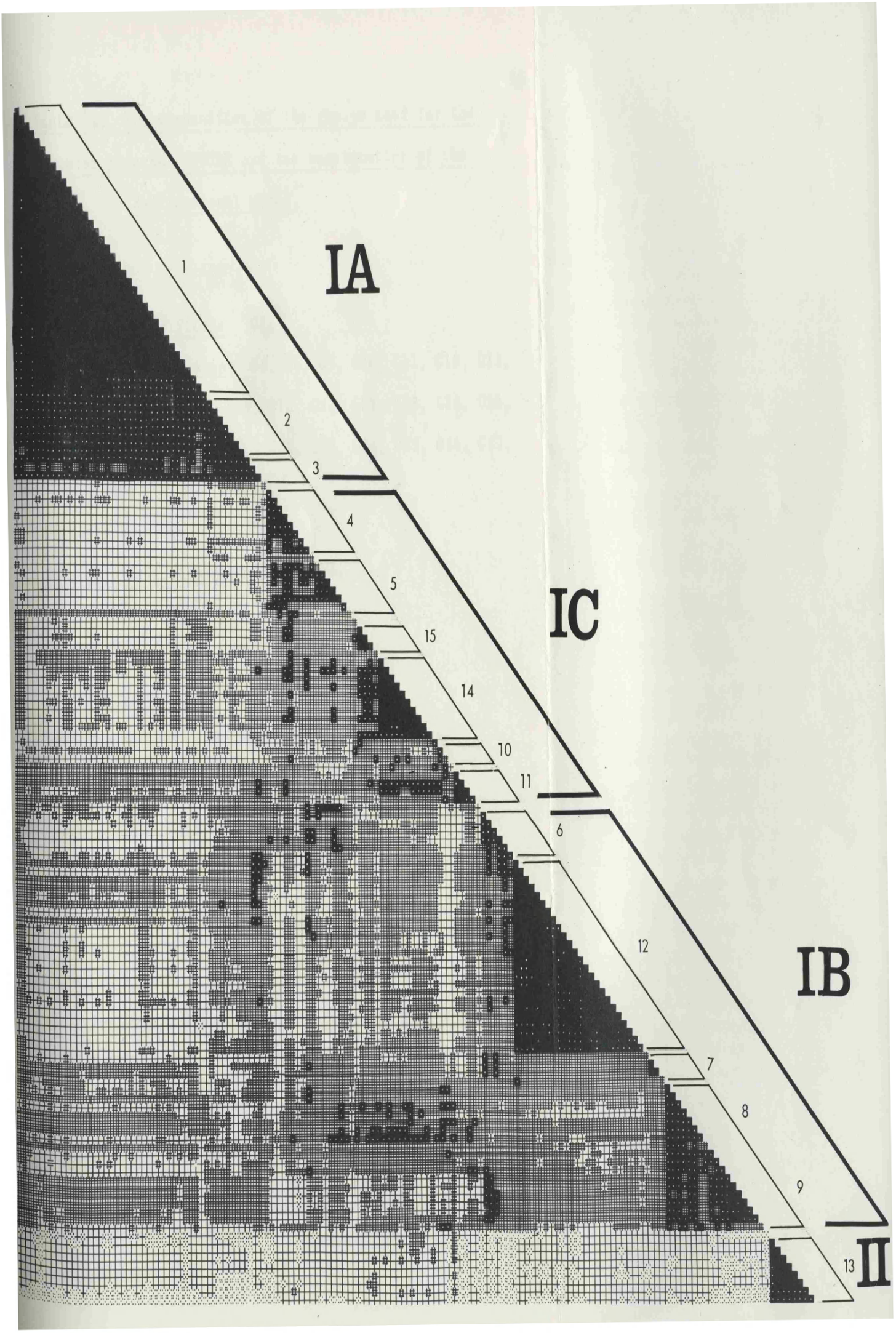

IA
IC
IB
II
1
2
3
4
5
15
14
10
11
6
12
7
8
9
13

TABLE 14. The composition of the groups used for the computer program IGROUPS and the construction of the 3-dimensional model.

GROUP 1.

Listeria denitrificans G1.

Listeria monocytogenes G5, G6, G7, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24, G25, G26, G27, G28, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40.

Listeria sp. G4.

Erysipelothrix rhusiopathiae G191.

GROUP 2.

Listeria grayi G2, G3, G42, G47.

Listeria murrayi G44, G45, G46.

GROUP 3.

Listeria monocytogenes G15a, G16a.

Listeria sp. G8.

GROUP 4.

Lactobacillus acidophilus G60.

Lactobacillus bulgaricus G64.

Lactobacillus delbrueckii G68, G81.

Lactobacillus helveticus G72.

Lactobacillus jugurt G73, G74.

Lactobacillus lactis G75.

Lactobacillus sp. G80.

TABLE 14. (continued).

GROUP 5.

Lactobacillus brevis G62.

Lactobacillus buchneri G63.

Lactobacillus cellobiosus G67.

Lactobacillus fermentum G69, G70, G71.

Lactobacillus leichmannii G76.

GROUP 6.

Lactobacillus casei G65, G66.

Lactobacillus plantarum G77.

Lactobacillus salivarius G78, G79.

Erysipelothrix rhusiopathiae G189.

GROUP 7.

Lactobacillus mali G142, G143, G144.

GROUP 8.

Streptococcus pyogenes G120.

Streptococcus agalactiae G121.

Streptococcus equi G122.

Streptococcus durans G124.

Streptococcus sp. (E) G129.

Streptococcus sp. (F) G130.

Streptococcus sp. (G) G131.

Streptococcus sp. (H) G132.

Streptococcus sp. (K) G1330, G133T.

Streptococcus sp. (N) G136.

TABLE 14. (continued).

GROUP 9.

Streptococcus bovis G123.

Streptococcus faecalis G125.

Streptococcus faecium G126.

Streptococcus liquifaciens G127.

Streptococcus zymogenes G128.

Streptococcus sp. (Q) G134.

Streptococcus sp. G138.

Streptococcus equinus G140.

GROUP 10.

Streptococcus sp. (M) G135.

Streptococcus sp. (O) G137.

Streptococcus uberis G139.

Streptococcus suis G141.

GROUP 11.

Streptococcus suis G196, G197, G198, G199, G200.

GROUP 12.

Microbacterium thermosphactum G86, G87, G88, G89, G90, G91, G92, G93, G94, G95, G96, G97, G98, G99, G100, G101, G102, G103, G104, G105, G106, G107, G108, G109, G110.

GROUP 13.

Kurthia zopfii G111, G112, G113, G114, G115, G116, G117, G118, G119.

TABLE 14. (continued).

GROUP 14.

Erysipelothrix rhusiopathiae G183, G184, G185, G186, G187, G188, G190, G192, G193, G194, G195.

GROUP 15.

Gemella haemolysans G82, G83, G84.

Neisseria haemolysans G85.

was possible to produce a table of results which distinguished between the 14 clusters from cluster I (TABLE 15). A further table was also produced which contained results which distinguished between the following, larger groups- IA1, IA2 and IA3 / IC4 and IC5 / IB6, IB7 and IB12 / IB8 and IB9 / IB10 and IB11 / IC14 and IC15. Unfortunately it was not possible to find discriminating characters for each of the main divisions in the dendrogram, but the characters set out in TABLES 15 and 16 may prove helpful in identification.

A further set of data was also compared using the program ITBNCLST, the strains included were the same as those in Dendrogram C (Figure 4), but with the addition of the four strains of Propionibacterium (cluster IB16) and the six strains from cluster IIB17 (a total of 159 OTUs). Dendrogram D (Figure 6) and Similarity Matrix D (Figure IV in Appendix III) were produced.

Using the inter- and intra- group similarities (TABLE 17) produced by the program IGROUPS for the clusters in Dendrogram C (Figure 4), it was possible to construct a model with polystyrene balls and wire to illustrate the relationships between Groups 1 to 12, 14 and 15. Group 13 (K. zopfii) was too dissimilar from the remaining groups to be included to any advantage. The model can be seen in Figure 7 .

The similarities were used directly to produce the model, but it would have been more accurate to calculate the taxonomic distances using the following formula :-

$$d = \sqrt{1-S}$$

where d is the taxonomic distance and S the similarity.

TABLE 15 . Characters which distinguish between Listeria (L. monocytogenes, L. grayi and L. murrayi -- Clusters IA1 and IA2) and the other eleven clusters (excluding K. zopfii) included in Figure 4 (IA4, IA5, IA6, IA7, IA8, IA9, IA10, IA11, IA12, IA14 and IA15).

Characters.	Listeria	Lactobacillus (Thermobacterium)	Lactobacillus (Betabacterium)	Lactobacillus (Streptobacterium)	Lactobacillus mali	Streptococcus I	Streptococcus II	Streptococcus III	Streptococcus IV	M.thermosphactum	Erysipelothrix	G.haemolysans

TABLE 16. Characters which distinguish between Listeria (clusters 1,2,3), 16 strains of Lactobacillus (clusters 4,5), the remaining 9 strains of Lactobacillus and M.thermosphactum (clusters 6,7,12), 19 strains of Streptococcus (clusters 8,9), the remaining 9 strains of Streptococcus (clusters 10,11) and Erysipelothrix and Gemella (clusters 14,15).

Characters	Clusters. 1,2,3	4,5	6,7,12	8,9	10,11	14,15
inhibition by K. thiocyanate 2.5-4.5%	-	+	+	+	+	+
long rods	-	+	+ 18/34	-	-	-
cocci	- 45/47	-	- 33/34	+	+	- 14/15
beaded Gram stain	-	+	+	-	-	-
motile	+ 38/47	- 15/16	- 31/34	+	+ 4/9	-
reduction of 0.01%	+	+ 9/16	+ 32/34	+	- 8/9	+ 10/15
K. tellurite 0.025%	+	- 9/16	+ 21/34	+ 18/19	-	+ 10/15
inhibition by 0.01% tetrazolium	- 46/47	+	+	+ 10/19	+ 8/9	-
arginine deamination ($-O_2$)	- 46/47	- 10/16	-	+ 15/19	+ 8/9	+ 11/15
aesculin hydrolysis	+	- 14/16	+ 33/34	+ 15/19	+ 8/9	-
phosphatase activity	+ 46/47	+ 9/16	+	+ 17/19	- 8/9	+ 10/15
salicin fermentation	+	- 13/16	+	+ 16/19	+ 6/9	-
sorbitol "	- 44/47	-	+ 31/34	- 13/19	- 8/9	-
No. of strains	47	16	34	19	9	15

N.B. fractions indicate proportion of +/- results, otherwise all strains reacted in the same way.

Figure 6. Dendrogram D.

(159 OTUs)

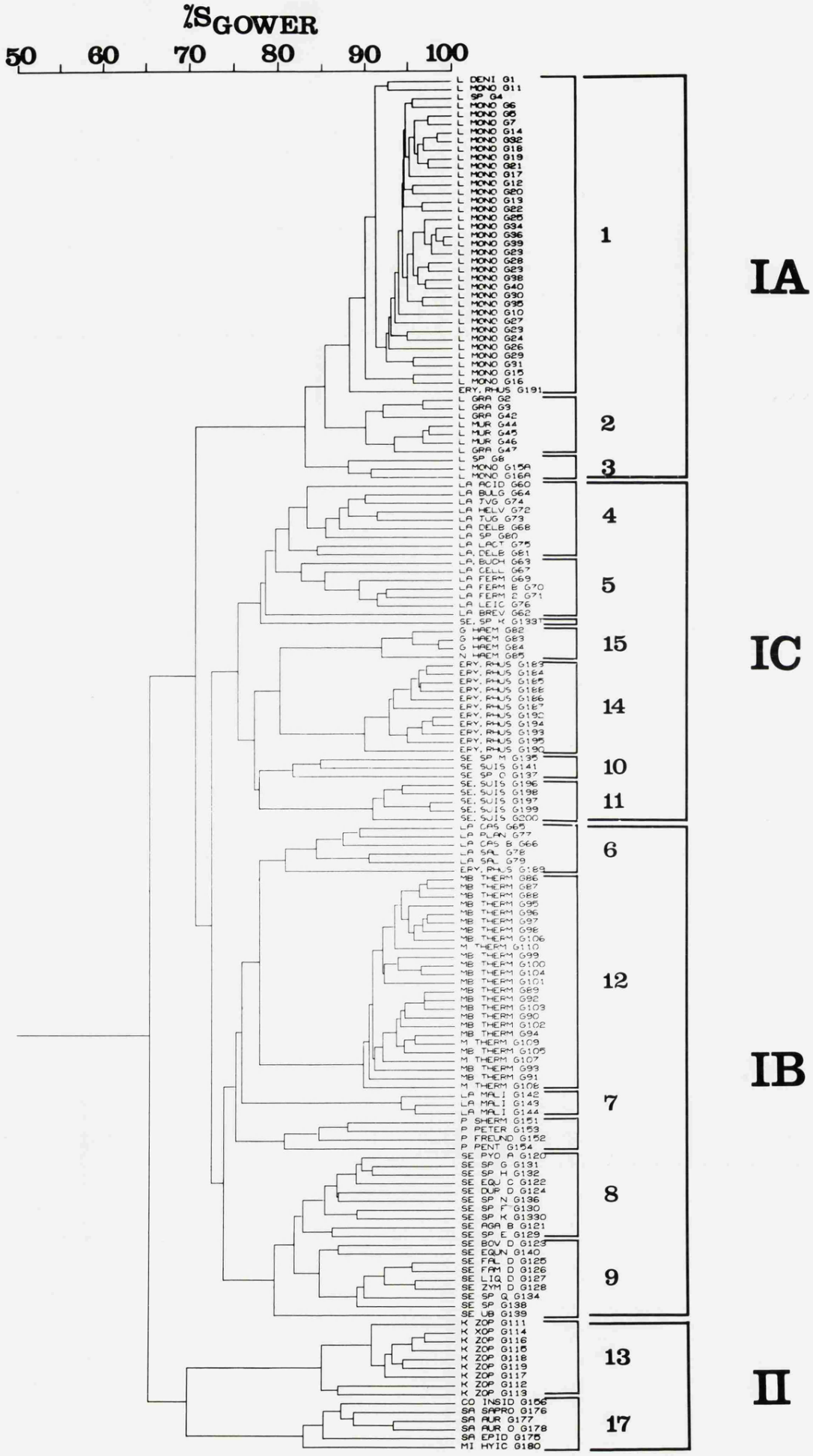
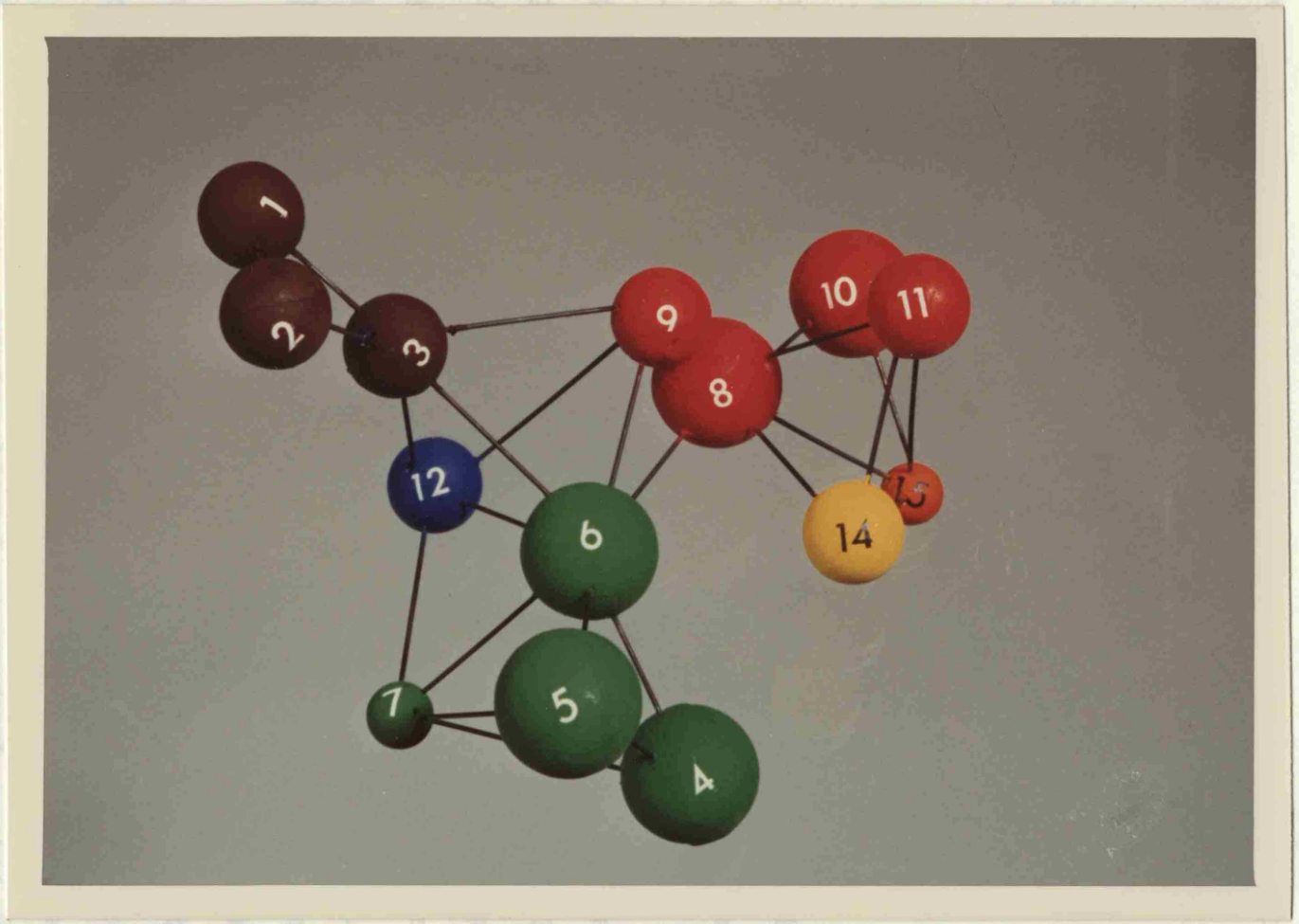

%SGOWER
50
60
70
80
90
100
L DENI G1
L MONO G11
L SP G4
L MONO G6
L MONO G5
L MONO G7
L MONO G14
L MONO G32
L MONO G18
L MONO G19
L MONO G21
L MONO G17
L MONO G12
L MONO G20
L MONO G13
L MONO G22
L MONO G25
L MONO G34
L MONO G36
L MONO G39
L MONO G29
L MONO G28
L MONO G23
L MONO G38
L MONO G40
L MONO G30
L MONO G35
L MONO G10
L MONO G27
L MONO G33
L MONO G24
L MONO G26
L MONO G29
L MONO G31
L MONO G15
L MONO G16
ERY. RHUS G191
L GRA G2
L GRA G3
L GRA G42
L MUR G44
L MUR G45
L MUR G46
L GRA G47
L SP G8
L MONO G15A
L MONO G16A
LA ACID G60
LA BULG G64
LA JVG G74
LA HELV G72
LA TUG G73
LA DELB G68
LA SP G80
LA LACT G75
LA. DELB G81
LA. BUCH G63
LA CELL G67
LA FERM G69
LA FERM B G70
LA FERM 2 G71
LA LEIC G76
LA BREV G62
SE. SP K G133
G HAEM G82
G HAEM G83
G HAEM G84
N HAEM G85
ERY. RHUS G183
ERY. RHUS G184
ERY. RHUS G185
ERY. RHUS G188
ERY. RHUS G186
ERY. RHUS G187
ERY. RHUS G192
ERY. RHUS G194
ERY. RHUS G193
ERY. RHUS G195
ERY. RHUS G190
SE SP M G135
SE SUIS G141
SE SP O G137
SE. SUIS G196
SE. SUIS G198
SE. SUIS G197
SE. SUIS G199
SE. SUIS G200
LA CAS G65
LA PLAN G77
LA CAS B G66
LA SAL G78
LA SAL G79
ERY. RHUS G189
MB THERM G86
MB THERM G87
MB THERM G88
MB THERM G95
MB THERM G96
MB THERM G97
MB THERM G98
MB THERM G106
M THERM G110
MB THERM G99
MB THERM G100
MB THERM G104
MB THERM G101
MB THERM G89
MB THERM G92
MB THERM G103
MB THERM G90
MB THERM G102
MB THERM G94
M THERM G109
MB THERM G105
M THERM G107
MB THERM G93
MB THERM G91
M THERM G108
LA MALI G142
LA MALI G143
LA MALI G144
P SHERM G151
P PETER G153
P FREUND G152
P PENT G154
SE PYO A G120
SE SP G G131
SE SP H G132
SE EQU C G122
SE DUR D G124
SE SP N G136
SE SP F G130
SE SP K G1330
SE AGA B G121
SE SP E G129
SE BOV D G123
SE EQUN G140
SE FAL D G125
SE FAM D G126
SE LIQ D G127
SE ZYM D G128
SE SP Q G134
SE SP G138
SE UB G139
K ZOP G111
K XOP G114
K ZOP G116
K ZOP G115
K ZOP G118
K ZOP G119
K ZOP G117
K ZOP G112
K ZOP G113
CO INSID G156
SA SAPRO G176
SA AUR G177
SA AUR O G178
SA EPID G175
MI HYIC G180
1
2
3
4
5
15
14
10
11
6
12
7
8
9
13
17
IA
IC
IB
II

TABLE. 17. Mean Inter- and Intra- Group Similarities from the IGROUPS Computer Program.

Group No.															
1	.929														
2	.852	.919													
3	.825	.834	.886												
4	.675	.682	.689	.837											
5	.661	.676	.696	.793	.836										
6	.685	.729	.749	.752	.771	.841									
7	.707	.687	.709	.732	.741	.769	.944								
8	.717	.703	.719	.733	.749	.738	.735	.839							
9	.717	.740	.755	.672	.719	.778	.740	.805	.879						
10	.689	.657	.688	.720	.707	.690	.719	.790	.741	.802					
11	.731	.725	.762	.742	.709	.709	.691	.747	.701	.772	.920				
12	.694	.728	.776	.701	.708	.776	.753	.720	.736	.678	.731	.915			
13	.611	.580	.600	.631	.640	.582	.661	.632	.595	.634	.595	.614	.893		
14	.702	.699	.749	.755	.774	.725	.728	.773	.713	.749	.786	.723	.684	.930	
15	.690	.664	.703	.766	.757	.726	.731	.775	.713	.764	.735	.716	.673	.800	.940

Figure 7. Photograph of a 3-dimensional model illustrating the taxonomic relationship between Groups 1 - 12, 14 and 15 as detailed in TABLE 14.



Only three sizes of polystyrene balls were available, and it was, therefore, necessary to impose arbitrary divisions upon the intra-group similarities to produce three "size" categories, but this was greatly facilitated by the fact that there were three naturally occurring "size" groups. The three groups, expressed as:-

(1 - intra-group similarity) x 100,

are as follows:-

5.6 - 8.5	groups	1, 2, 7, 11, 12, 14, 15.
10.7 - 12.1	groups	3, 9.
15.9 - 19.8	groups	4, 5, 6, 8, 10.

Accordingly, the small, medium or large balls were used, which at the scale employed, corresponded well with the scale for the inter-group distances.

Group 1 (Listeria monocytogenes), group 2 (L. grayi and L. murrayi) and group 3 (G8, G15a and G16a) are all shown in black in the model and form a tight cluster. The four groups of Lactobacillus (4, 5, 6 and 7) are all shown in green, and form another tight cluster. The streptococci (groups 8, 9, 10 and 11), shown in red, together with Erysipelothrix (group 14) which is yellow, and Gemella (group 15) in orange, form a third cluster. These three clusters are all approximately the same taxonomic distance from the final group (12), which contains the strains of M. thermosphactum and is shown in blue. This, M. thermosphactum, cluster lies in a central position in the model, and appears to be a link between the three main clusters detailed above.

Reproducibility.

The reproducibility of results in numerical taxonomic studies has received increasing attention in recent years. The effect of errors in microbiological tests, on numerical taxonomic similarities, has been discussed by Sneath and Johnson (1972). Because of this increasing interest in reproducibility it was decided to repeat the tests used in the present study on a proportion of the strains. Since 46 strains had already been tested at both 30°C and 35°C (for reasons discussed earlier) it was decided to take 43 of these strains, to split 5 of them and treat them as separate strains, and to repeat the tests on them at 35°C.

The variation between the three sets of results was calculated. The effect of incubation at different temperatures was investigated by comparing the sets of results obtained simultaneously at 30°C and 35°C, the average error was 11.06%. In this instance the tests were inoculated and read simultaneously, and the same batches of media used. The error is, therefore, attributable to the effect of the temperature of incubation, human error in performing the tests and reading the results and any intrinsic variability of the individual strains. The two sets of data, obtained from tests performed at 35°C, but on different occasions, using different batches of media, showed an average error of 15.74%. The five pairs of split strains were found to show between 88.5% S and 92.7% S to their respective partners (see TABLE 18), giving an average reproducibility of 92.7%, or an average error of 7.3%.

The variation observed is greater than the 3.42% reported

TABLE 18. Similarities observed between duplicate cultures of five strains of bacteria, tested simultaneously.

Collection No.	Strain.	Percentage similarity.
G 30	L. monocytogenes	92.7
G 45	L. murrayi	96.2
G 65	Lactobacillus casei	90.0
G 120	S. pyogenes	88.5
G 190	E. rhusiopathiae	95.1

by Sneath and Johnson (1972), or the value of 6.9% observed by Lapage, Bascomb, Willcox and Curtis (1970). It is clear that among the present tests some were very good, while others were bad, and showed a very low level of reproducibility.

Good tests included the modified "O" and "F" test; the hydrolysis of xanthine, tyrosine, Tween 80 and æsculin; the production of indole; sulphatase and oxidase activity; growth under an atmosphere of hydrogen; reduction of nitrate; inhibition by chloramphenicol, chlortetracycline. erythromycin, oleandomucin, oxytetracycline, penicillin and tetracycline; growth at the more intermediate temperatures (10°C, 20°C, 30°C

and 35°C). Bad tests included growth at the more extreme temperatures (5°C, 45°C and 50°C). growth in the presence of sodium chloride (6.5%w/v and 10%w/v) and nitrite; inhibition by potassium thiocyanate; the production of ammonia from peptone; hydrogen sulphide production and many of the morphological characters (e.g., watery or butyrous consistency, low convex, smooth/rough colonies, the presence of cocci, coccobacilli or short rods).

The reproducibility of the 32 morphological characters was compared with that for the remaining test, and all of the tests, for the two sets of comparisons (see TABLE 19).

TABLE 19. Comparison of the percentage error observed for the morphological tests, the remaining tests and all tests.

Comparison.	PERCENTAGE ERROR		
	Morphological characters.	Remaining characters.	Total characters.
30°C / 35°C (simultaneous, same batches of media.)	15.8	9.6	11.0
35°C / 35°C (different times and different batches of media.)	18.0	15.0	15.7

The morphological characters are, in general, more subjective than the biochemical and physiological characters, this probably accounts for the higher error values obtained, and clearly, no numerical taxonomic survey should be too heavily weighted towards morphological characteristics. Another source of error is evaporation from liquid media and the resultant increase in concentration of any inhibitory substances. This, at least in part, accounts for the poor reproducibility observed for growth on the presence of 6.5%w/v and 10%w/v sodium chloride, and other workers have commented upon the unreliability of these two tests. The problems involved in the assessment of growth at various temperatures are obvious; near the optimun for a particular strain fluctuations will have a minimal effect upon the growth rate, but nearer the extremes a fluctuation of $1^{o}C$ could easily alter the result. Listeria, for example, has a limit of growth at approximately $45^{o}C$, some workers have reported growth, others no growth at this temperature.

The range of percentage errors obtained in the two comparisons can be seen in Figures 8 and 9. Over half of the tests in the $30^{o}C$ / $35^{o}C$ comparison exhibited errors of less than 8.7%. 49 of these were under 4.4%, and 26 showed 100% reproducibility. For the two sets of results at $30^{o}C$, 70 of the 143 tests had errors of 9.3% or less, of these 49 were less than 4.7%, and 18 tests showed complete reproducibility. The rather high 'over all' level of error, therefore, is clearly due to the bad tests, a few of which showed as much as a 50% error.

Clearly there are good and bad tests with regard to

Figure 8. Histogram illustrating the number of characters (N) which exhibit the various levels of error (% E) in the 35°C / 35°C comparison.

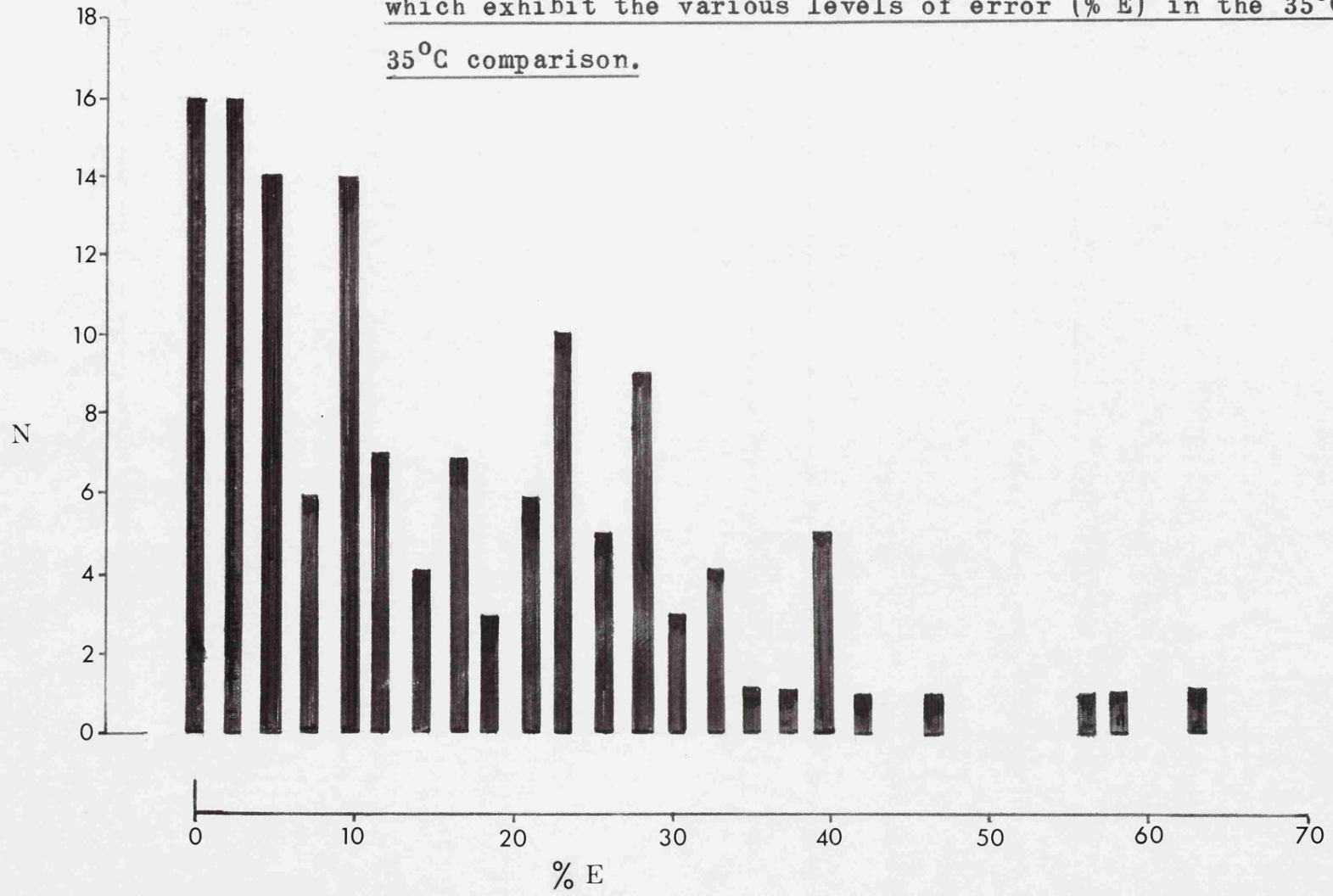
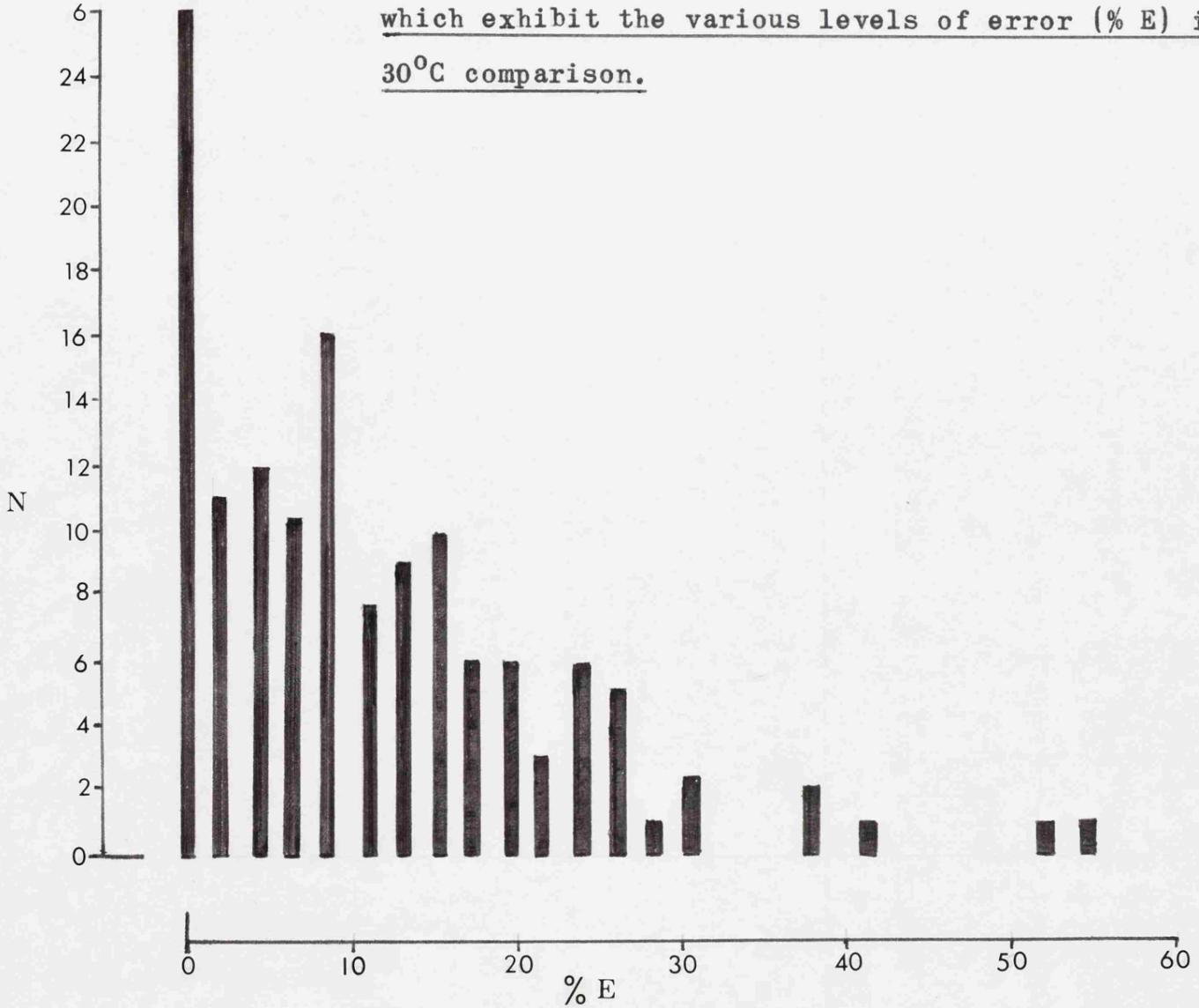


Figure 9. Histogram illustrating the number of characters (N) which exhibit the various levels of error (% E) in the 35°C / 30°C comparison.



error, the concern over the reproducibility of results in numerical taxonomy is well founded, and consideration should be given to such error levels. The importance of performing all of the tests for a single character at the same time, using the same batch of medium, in order to minimize the error as much as possible, is clearly reinforced.

II. Serological Results.

The homologous titres of the prepared antisera were obtained by tube agglutination as described in the serological methods section and are shown in TABLE 20.

a) Somatic (O) Antisera.

The somatic antisera, both prepared and purchased, were tested against HCl extracts of all the strains listed in TABLE 10 in the methods section. Unfortunately, several HCl extracts of Streptococcus faecium (group D), Streptococcus sp. (group K) and Streptococcus sp. (group Q) reacted weakly, or not at all, with the antisera obtained for their respective serological groups. It was found that HCl extracts of S. faecalis did react well with the group D antiserum, and this bacterium was used for the remainder of the tests. Tests for the group K and group Q antisera and group K HCl extracts were not performed. The other streptococcal antisera were specific and in most cases only reacted with the homologous HCl extract, and that of Corynebacterium poinsettiae (which reacted with all the antisera). The results for the streptococci are summarised in TABLE 21 below.

TABLE 20. Homologous titres of the prepared antisera.

Strain No.	Bacterium	Antibody type	Titre
G 32	Listeria monocytogenes type 1a	'O'	⩾1,024
"	" " " "	'H'	256
G 22	" " " 2	'O'	1,024
"	" " " "	'H'	1,024
G 29	" " " 3a	'O'	⩾1,024
"	" " " "	'H'	512
G 30	" " " 4a	'O'	⩾1,024
"	" " " "	'H'	⩾1,024
G 42	" grayi	'O'	*
G "	" "	'H'	32,768
G 44	" murrayi	'O'	1,024
G 43	" denitrificans	'O'	512
G 64	Lactobacillus bulgaricus	'O'	1.024
G 65	" casei	'O'	⩾1.024
G 82	Gemella haemolysans	'O'	⩾1,024
G 104	Microbacterium thermosphactum	'O'	256
G 158	Corynebacterium poinsettiae	'O'	*
G 168	Kurthia zopfii	'O'	1,024
G 190	Erysipelothrix	'O'	⩾1,024

* Antisera against these bacteria were not obtained until late in the inoculation program when the ring precipitin test was used for testing, intense lines were produced, however, within 10 minutes.

TABLE 21. Results of the ring precipitin tests with the Streptococcus antisera. (Only positive results are given).

ANTISERUM AGAINST:-			ANTIGEN:-		
G No.	Bacterium	Serological group	G No.	Reaction	C. poinsettiae
120	S. pyogenes	A	120	$+++_1$	$+_3$
121	S. agalactiae	B	121	$+++_1$	$+_3$
			TS II	$+_2$	
122	S. equi	C	122	$+++_1$	$+_2$
125	S. faecalis	D	125	$+++_1$	$+_1$
129	Streptococcus sp.	E	129	$+++_1$	$+_2$
130	Streptococcus sp.	F	130	$+++_1$	$++_2$
131	Streptococcus sp.	G	131	$+++_1$	$+_3$
132	Streptococcus sp.	H	132	$+++_1$	$+++_2$
48	Streptococcus sp.	L	48	$+++_1$	$++_2$
			42	$+_2$	
			44	$+_1$	
			64	$+_1$	
			104	$+_2$	
			168	$++_2$	
135	Streptococcus sp.	M	135	$+++_1$	$++_3$
136	S. lactis	N	136	$+++_1$	$++_3$
137	Streptococcus sp.	O	137	$+++_1$	$++_3$
49	Streptococcus sp.	P	49	$+++_1$	$++_3$
50	Streptococcus sp.	R	50	$+++_1$	$++_3$
51	Streptococcus sp.	S	51	$+++_1$	$++_3$

See following page for definition of the symbols.

TABLE 21. (continued).

+	weak fuzzy line.
++	fairly distinct line.
+++	very intense line.
1	result after 10 minutes.
2	" " 30 minutes.
3	" " 60 minutes.

The results of the ring precipitin tests in which the prepared antisera were tested against the HCl extracts are shown in TABLE 22.

Two of the Listeria monocytogenes '0' antisera (serological types 2 and 4a) were non-specific, and reacted with the majority of the HCl extracts, and similarly, the L. grayi '0' antiserum was non-specific, and it reacted with all of the HCl extracts with which it was tested. The other antera were more specific. The '0' antisera against L. murrayi and K. zopfii reacted with only their homologous HCl extracts and that of Corynebacterium poinsettiae. The '0' antiserum against this latter bacterium was specific, and only reacted with the homologous HCl extract. Other antisera reacted with their homologous extracts, that of C. poinsettiae and a further one to five extracts.

All positive reactions in the ring precipitin test were further investigated using double gel diffusion. The antisera against the streptococci only produced lines of precipitation with their homologous HCl extracts. In all but three

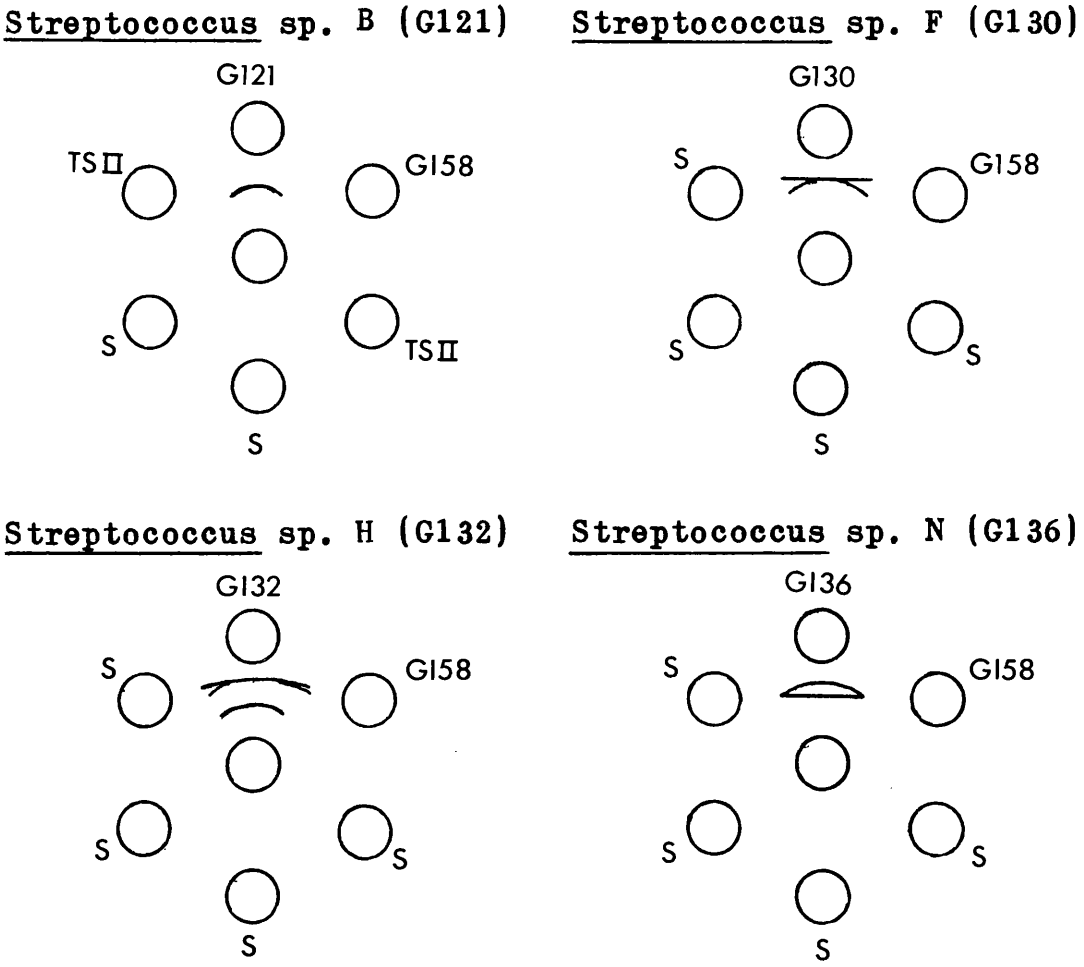
TABLE 22. Results of the ring precipitin test with prepared antisera and all HCl extracts.

ANTIGENS.		ANTISERA (G numbers of the bacteria used).													
G No.	Bacterium.	32	22	29	30	44	43	42	64	65	82	104	158	168	190
32	L. monocytogenes 1a	$+++_1$	$+++_1$	$+++_1$	$+++_2$	-	-	$++_2$	-	-	-	-	-	-	-
22	L. monocytogenes 2	$+++_1$	$+++_1$	$++_2$	$++_1$	-	-	$++_2$	-	-	-	-	-	-	-
29	L. monocytogenes 3a	$++_2$	$+++_1$	$+++_1$	$++_1$	-	-	$++_2$	-	-	-	-	-	-	-
30	L. monocytogenes 4a	-	$+++_2$	-	$+++_2$	-	-	$++_2$	-	-	-	-	-	$+++_1$	-
44	L. murrayi	-	$+++_1$	-	$+++_2$	$+++_1$	$+++_2$	$+++_2$	-	-	-	-	-	-	-
43	L. denitrificans	-	$+++_2$	-	$+++_2$	-	$+++_2$	$++_2$	-	-	-	-	-	-	-
1	L. denitrificans	-	$+++_2$	-	-	-	-	-	-	-	-	-	-	-	-
42	L. grayi	-	$+++_1$	-	$+++_2$	-	-	$+++_2$	-	-	-	-	-	-	-
64	Lactobacillus bulgaricus	-	$+++_2$	$+_2$	$+++_2$	-	-	$++_2$	$+++_2$	$++_3$	-	-	-	-	-
65	Lactobacillus casei	-	$+++_1$	-	$+++_2$	-	-	$++_2$	-	$+++_1$	-	-	-	-	-
82	G. haemolysans	-	-	-	-	-	-	$++_2$	-	-	$+++_1$	-	.	-	-
104	M. thermosphactum	-	$+++_1$	-	$+++_2$	-	-	$++_2$	-	-	-	$+++_3$	-	-	-
158	C. poinsettiae	$+++_1$	$+++_1$	$+++_1$	$+++_1$	$+++_1$	$+++_1$	$+++_1$	$+++_1$	$+++_1$	$++_1$	$+++_1$	$+++_1$	$+++_1$	$+++_1$
168	K. zopfii	-	$+++_3$	-	$+++_3$	-	-	$++_2$	-	$+_2$	-	-	-	$+++_1$	-
190	E. rhusiopathiae	-	$+++_2$	-	$+++_2$	-	-	-	-	-	-	-	-	-	$+++_1$
120	S. pyogenes (A)	-	$++_2$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
121	S. agalactiae (B)	-	$+++_2$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
122	S. equi (C)	-	$+++_3$	-	$++_3$	-	-	.	-	-	-	-	-	-	-
125	S. faecalis (D)	-	$+++_3$	-	-	-	-	.	-	-	-	-	-	-	-
129	Streptococcus sp. (E)	-	$+++_1$	-	$+_1$	-	-	.	-	-	-	-	-	-	-
130	" " (F)	-	$++_3$	-	$+_1$	-	-	.	-	-	-	-	-	-	-
131	" " (G)	-	$+_2$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
132	" " (H)	-	$+++_1$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
48	" " (L)	-	$+++_2$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
135	" " (M)	-	$+++_2$	-	$+++_3$	-	-	.	-	-	$+_1$	$+_3$	-	-	-
136	" " (N)	-	$+++_2$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
137	" " (O)	-	$+++_1$	-	$++_1$	-	-	.	-	-	-	-	-	-	-
49	" " (P)	-	$+++_3$	-	$+_1$	-	$+_2$	.	$+_2$	-	-	-	-	-	$+_2$
134	" " (Q)	-	$+++_1$	-	$++_1$	-	-	.	-	-	-	-	-	-	-
50	" " (R)	-	$+++_2$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
51	" " (S)	-	$+++_2$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
TS I		-	$+++_3$	-	$++_2$	-	-	.	-	-	-	-	-	-	-
TS II		-	$+++_1$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-
TS III		-	$+++_1$	-	$+++_2$	-	-	.	-	-	-	-	-	-	-

SYMBOLS as in TABLE 21, . not tested.

instances only a single line was produced, but two or three lines resulted from the reactions with streptococcal antisera against serological groups F, M, and N (see Figure 10 below).

Figure 10. Results of the double gel diffusion tests with streptococcal antisera against serological groups B, F, M and N.



G158 C. poinsettiae S saline control

The double gel diffusion results for the prepared 'O' antisera are shown in TABLE 23.

TABLE 23. Results of the double gel diffusion test for the prepared antisera.

ANTISERA. G No.	Bacterium	Ring Precipitin Test Positive G No.	Bacterium	Double Gel Diffusion Result.
32	L. monocytogenes 1a		homologous	3 lines
		22	L.monocytogenes 2	3 lines as homologous
		29	L.monocytogenes 3a	-
		158	C.poinsettiae	-
29	L. monocytogenes 3a		homologous	1 line
		22	L.monocytogenes 2	-
		32	L.monocytogenes 1a	-
		64	L.bulgaricus	-
		158	C.poinsettiae	-
			TS III	-
22	L. monocytogenes 2		homologous	1 line
		32	L.monocytogenes 1a	1 line as G22 + 1 faint line
		(29, 30, 42, 43, 44, 64, 65, 104, 190, 125, 129, 130, 131, 48, 135, 136, 137, 134, 50, 51, TSII, TS III)		all gave 1 faint line as G32.
		(158, 168, 120, 121, 122, 49, TS I)		-
30	L. monocytogenes 4a		homologous	-
			(see TABLE 22)	-

TABLE 23. (continued).

ANTISERA G No.	Bacterium	Ring Precipitin Test Positive G No.	Bacterium	Double Gel Diffusion Results.
44	L. murrayi		homologous	-
		158	C. poinsettiae	-
43	L. denitrificans		homologous	1 faint line
		44	L. murrayi	-
		135	Streptococcus sp.(M)	-
		158	C. poinsettiae	-
64	Lactobacillus bulgaricus		homologous	1 line
		49	Streptococcus sp.(P)	-
		158	C. poinsettiae	-
65	Lactobacillus casei		homologous	3 lines
		64	L. bulgaricus	all share 1 faint line with G 65.
		132	Streptococcus sp.(H)	
		158	C. poinsettiae	
		168	K. zopfii	
82	Gemella haemolysans		homologous	1 line
		135	Streptococcus sp.(M)	-
		158	C. poinsettiae	-
104	Microbacterium thermosphactum		homologous	-
		135	Streptococcus sp.(M)	-
		158	C. poinsettiae	-
158	Corynebacterium poinsettiae		homologous	-

TABLE 23. (continued).

ANTISERA G No.	Bacterium	Ring Precipitin Test Positive G No.	Bacterium	Double Gel Diffusion Results.
168	Kurthia zopfii		homologous	1 faint line
		30	L. monocytogenes 4a	as homologous + 1 strong line
		158	C. poinsettiae	as homologous + 1 faint line
190	Erysipelothrix rhusiopathiae		homologous	1 strong line
		49	Streptococcus sp.(P)	-
		158	C. poinsettiae	-

- no visible reaction was observed.

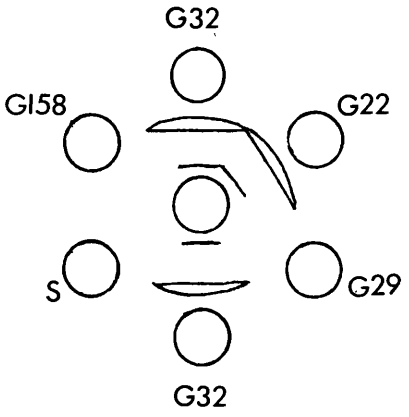
See Figure11for illustrations of those antisera which reacted with other antigens as well as the homologous.

The double gel diffusion results for those prepared 'O' antisera which reacted with other HCl extracts as well as the homologous, are illustrated in Figure 11; the complete set of results for the non-specific antiserum against L. monocytogenes serotype 2 (G 22) are not illustrated, but the lines produced with HCl extracts other than the homologous were all shown to be identical to the fainter outer line produced against L. monocytogenes serotype 1a (G 32). This latter organism also produced a more intense line, closer to the central well, identical to the line produced with the homologous HCl extract (see Figure 11).

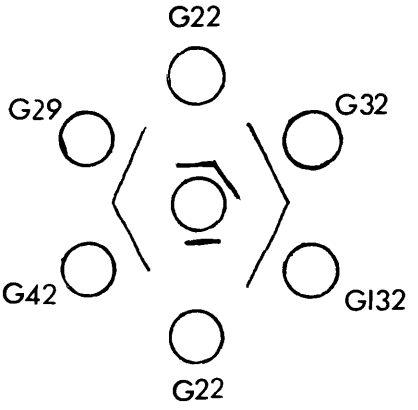
The antiserum against G22 (diluted by half in saline) was absorbed with L. murrayi 'O' antigens and retested by double gel diffusion and the ring precipitin test against all the HCl extracts with which it had previously shown a reaction. In the ring precipitin test only the reactions with the homologous HCl extract, that of L. monocytogenes G32 (1a) and that of C. poinsettiae remained. Lines were no longer observed by double gel diffusion against any of the HCl extracts, even the homologous extract. The fact that the non-specific reaction with C. poinsettiae still remained when the other non-specific reactions had been removed by absorption, indicated that the substance(s) responsible for this reaction was distinct from the substance(s) involved in the non-specific reactions between the G 22 antiserum and the other HCl extracts. By double gel diffusion G32, serotype 1a, had previously produced two lines with the G 22 antiserum, one of these was identical to the non-specific reaction produced with all but the C. poinsettiae HCl extract, and the other was identical to the

Figure 11. Results of the double gel diffusion tests in which the prepared antisera reacted with other antigens as well as the homologous.

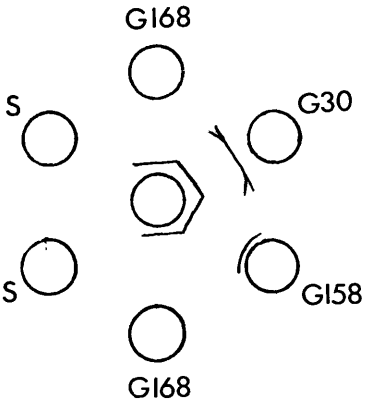
L. monocytogenes 1a (G32)



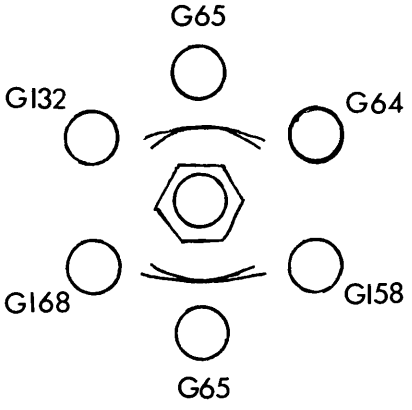
L. monocytogenes 2 (G22)

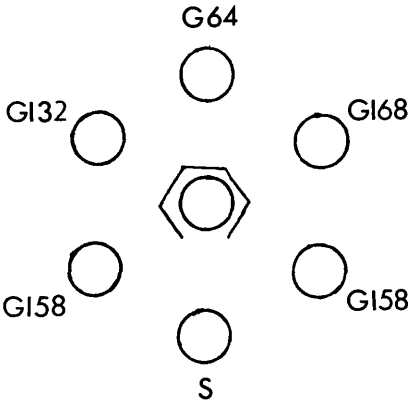


K. zopfii (G168)



Lactobacillus casei (G65)





G 29 L. monocytogenes 3a

G 42 L. grayi

G158 C. poinsettiae

G 64 Lactobacillus bulgaricus

G132 Streptococcus sp. group H

S saline control

line produced by the homologous reaction. The reaction in the ring precipitin test with the absorbed G 22 antisera and the G 32 antigens can be assumed to be due to the specific reaction observed in the double gel diffusion test prior to absorption, and this is compatible with the antigenic nature of the two strains, which have three distinct antigens in common (see TABLE 34, page 147).

The reaction of G 32 antisera with the homologous HCl extract and that of G 22 produced three identical lines with each extract (see Figure 11), and these probably correspond to their three shared 'O' antigens (see TABLE 34).

The antiserum against L. monocytogenes serotype 3a (G29) reacted with the serotypes 1a and 2 by the ring precipitin test, but failed to produce a line with any HCl extracts, other than the homologous, by the double gel diffusion test. The reactions with serotypes 1a and 2 may have been attributable to one of the 'O' antigens which these bacteria have in common, but because of the lack of reaction in the double gel diffusion test it was not possible to investigate this further.

The non-specific antiserum against G30 (serotype 4a) gave no reactions in the double gel diffusion test, and could not, therefore, be investigated further.

The relatively specific 'O' antisera produced against serotypes 1a and 3a (G 32 and G29) were tested by the ring precipitin test against HCl extracts of all the 36 strains of L. monocytogenes, Listeria sp. and L. denitrificans which fell within cluster IA1 in Figure 2. All extracts which gave a positive reaction with the G 32 antiserum were then tested by the gel diffusion test, see TABLE 24 .

TABLE 24. Results of the double gel diffusion and ring precipitin tests with antisera against L. monocytogenes serotypes 1a, 2 and 3a and HCl extracts of the members of cluster IA1 (see Figure 2), the L. monocytogenes cluster.

ANTIGENS. G No.	ANTISERA. 1a (G32) R.P.T.	1a (G32) D.G.D.	2 (G22) D.G.D.	3a (G29) R.P.T.
1	-	.	NS	$+++_3$
4	-	.	NS	$+++_3$
5 (1a)	$+++_1$	S	S	$+++_2$
6 (4b)	-	.	NS	$++_2$
7 (4)	$++_3$	-	NS	$+++_2$
10 (4a)	$++_3$	-	NS	$++_3$
11 (4a)	-	.	NS	$+_3$
12 (1)	$+++_1$	-	NS	$+++_3$
13 (1)	$+++_1$	S	NS	$++_2$
14 (1)	$+++_1$	S	NS + S	$++_2$
15	-	.	-	-
16	$++_3$	-	-	-
17 (4b)	$++_3$	-	NS	-
18	$+++_1$	S	NS	$+++_1$
19	-	.	NS	$++_1$
20	$+++_1$	S	S	$+++_1$
21	-	.	NS	$++_1$
22 (2)	$+++_1$	S	S	$++_1$
23	$+++_1$	S	NS	-
24 (3a)	$++_3$	-	NS	$+++_1$

TABLE 24. (continued).

ANTIGENS. G No.	ANTISERA. 1a (G32) R.P.T.	1a (G32) D.G.D.	2 (G22) D.G.D.	3a (G29) R.P.T.
25	$+++_3$	S(weak)	NS	-
26	$+++_1$	S	NS	$++_2$
27	$+++_1$	S	NS + S	$+++_3$
28	-	.	NS	$+++_3$
29 (3a)	$++_3$	-	-	$+++_1$
30 (4a)	-	-	NS	-
31	$+++_1$	S	S	$+++_3$
32 (1a)	$+++_1$	S	S	-
33	-	.	NS	-
34	-	.	NS	-
35	$++_3$	S(weak)	NS	$++_3$
36	$+++_1$	S	NS + S	$++_3$
37	$++_3$	S(weak)	NS	$+++_3$
38	$+++_3$	S	NS + S	$+_3$
39	-	.	NS	-
40	-	.	NS	-

. test not performed.

S specific line produced in D.G.D. test. NS non-specific.

D.G.D. double gel diffusion test.

R.P.T. ring precipitin test.

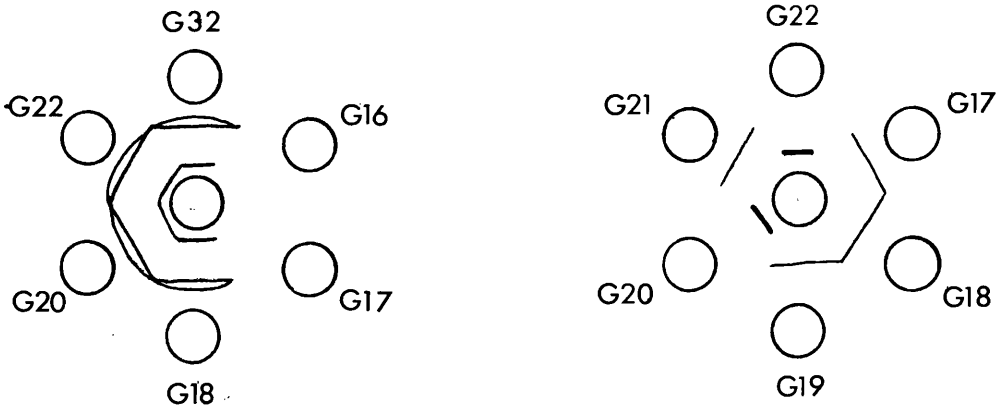
(1a) refers to serotype of the strain.

see TABLE 21. for symbols used for the R.P.T.

The antiserum against serotype 2 '0' antigens was tested against the same 36 strains from cluster IA1 by the double gel diffusion test. Some extracts reacted to produce the non-specific line discussed earlier, others produced the same specific line as the homologous HCl extract and others produced both lines (as G 32 serotype 1a). The results of all these tests are shown in TABLE 24 , and some of them are represented in Figure 12.

Figure 12. Results of some of the double gel diffusion tests which are detailed in TABLE 24.*

L. monocytogenes 1a (G32). L. monocytogenes 2 (G22).



* All the above bacteria (G16 to G23 plus G32) are all strains of L. monocytogenes.

b) Flagella (H) Antisera.

The flagella antisera produced against the four serotypes of L. monocytogenes (1a, 2, 3a and 4a) and L. grayi were tested by tube agglutination against their particulate 'H' antigen preparations, those of each other and those of a further eight strains which were included in the numerical taxonomic survey. These eight bacteria were L. murrayi (G44), L. denitrificans (G43), the four strains of L. monocytogenes which clustered most closely to the four above serotypes in the numerical taxonomic study (G13, G14, G31 and G35), Lactobacillus delbrueckii (G68) and Streptococcus faecalis (G125). Both of these latter two strains had demonstrated weak motility when tested in the numerical taxonomic study. The titres obtained are shown in TABLE 25.

The 'H' antisera were then absorbed with their corresponding 'O' antigens and retested with the various 'H' antigens by slide agglutination, and in some cases by tube agglutination (see TABLE 26 for the results).

Absorption of the four L. monocytogenes 'H' antisera with their homologous somatic antigens removed the cross-agglutination reactions with L. grayi, L. denitrificans, L. murrayi, Lactobacillus delbrueckii and S. faecalis, indicating that some form of non-specific or somatic reaction had been occuring. The reactions with the four L. monocytogenes strains G13, G14, G31 and G35 were not removed, but those of L. monocytogenes serotypes 1a and 2 with antisera against 3a and 4a no longer occurred. The L. grayi 'H' antiserum showed a low level of reaction with the L. monocytogenes and

TABLE 25. Results of the tube agglutination tests with unabsorbed 'H' antisera against L. monocytogenes (serotypes 1a, 2, 3a and 4a) and L. grayi, and 'H' antigens of several motile strains of bacteria.

ANTIGENS.		ANTISERA.				
		L.monocytogenes				L.grayi
G No.	Bacterium	1a/G32	2/G22	3a/G29	4a/G30	G42
32	L.monocytogenes 1a	258	512	8	128	64
22	L.monocytogenes 2	128	512	8	128	16
29	L.monocytogenes 3a	128	512	⩾1024	⩾1024	128
30	L.monocytogenes 4a	256	512	⩾1024	⩾1024	128
42	L.grayi	16	4	32	16	⩾1024
43	L.denitrificans	4	8	8	16	32
44	L.murrayi	8	2	2	16	⩾1024
13	L.monocytogenes	128	1024	⩾1024	⩾1024	64
14	"	128	512	⩾1024	⩾1024	64
31	"	128	⩾1024	⩾1024	⩾1024	64
35	"	128	128	256	⩾1024	128
68	L.delbrueckii	64	64	32	256	256
125	S.faecalis	256	128	128	128	256

TABLE 26. Results of the slide or tube agglutination tests with the absorbed 'H' antisera against L. monocytogenes (serotypes 1a, 2, 3a and 4a) and L. grayi, and antigens of several motile strains of bacteria.

ANTIGENS.		ANTISERA.				
		L.monocytogenes				L.grayi
G No.	Bacterium.	1a/G32	2/G22	3a/G29	4a/G30 *	G42 o
32	L.monocytogenes 1a	+++	+++	-	-	16
22	L.monocytogenes 2	+++	+++	-	-	4
29	L.monocytogenes 3a	+++	+++	+++	2048	32
30	L.monocytogenes 4a	+++	+++	+++	2048	32
42	L.grayi	-	-	-	-	32,768
43	L.denitrificans	-	-	-	-	-
44	L.murrayi	-	-	-	-	32,.768
13	L.monocytogenes	+++	+++	+++	2048	16
14	"	+++	+++	+++	2048	16
31	"	+++	+++	+++	2048	-
35	"	+++	+++	+++	4096	-
68	L.delbrueckii	-	-	-	-	-
125	S.faecalis	-	-	-	-	-

Slide Agglutination results:-

+++ very strong agglutination

- no agglutination

Tube Agglutination results:-

numbers refer to titres

- no titre

* the antiserum against G 30 was diluted 1 in 4.

o the antiserum against G 42 was diluted 1 in 2.

L. denitrificans strains before absorption, and reactions at 1 : 1024 or higher with its homologous antigen preparation and that of L. murrayi. After absorption, the serum (which was diluted by half in saline before absorption) showed a reduction in the titres with L. monocytogenes (1a, 2, 3a and 4a, G13 and G14) to 1 : 32 or less, and the reactions with L. denitrificans and L. monocytogenes G31 and G35 were completely removed. The reactions with the homologous antigen and that of L. murrayi, however, still remained, and both exhibited titres of 1 : 32,768.

The supernatants from the tube agglutination reactions of the absorbed L. grayi antiserum with L. grayi and L. murrayi antigens were tested by slide agglutination to detect any remaining ability to agglutinate these two antigens. The results are shown in TABLE 27, and indicate that both antigens were reacting with the same antibody.

It was also decided to test other interesting motile bacteria, L. denitrificans (G1), Kurthia zopfii(G168), Corynebacterium poinsettiae (G158) and TS III against the five absorbed 'H' antisera by slide agglutination. The results of these tests are shown in TABLE 28. K. zopfii, C. poinsettiae and TS III failed to react with any of the antisera, but L. denitrificans (G1) reacted with all four of the L. monocytogenes 'H' antisera.

TABLE 27. Results of the slide agglutination tests with 'absorbed' L. grayi 'H' antiserum (supernatants from the tube agglutination tests with L. grayi and L. murrayi) against L. grayi and L. murrayi 'H' antigens.

	DILUTIONS.		
	1 : 2 to 1 : 32	1 : 64	1 : 128 to 1 : 16,192
Supernatants from the tube agglutination test with L. grayi			
Reaction with L. grayi	+++	+	-
Reaction with L. murrayi	+++	+	-
Supernatants from the tube agglutination test with L. murrayi			
Reaction with L. grayi	+++	++	-
Reaction with L. murrayi	+++	++	-

+++ very strong agglutination, + weak agglutination,

++ moderate agglutination, - no agglutination.

TABLE 28. Results of the slide agglutination tests with the five absorbed Listeria 'H' antisera against L. denitrificans (G1), K. zopfii (G168), C. poinsettiae (G158) and TSIII.

ANTISERA.		ANTIGENS.			
G No.	Bacterium	G 1	G168	G158	TSIII
32	L. monocytogenes 1a	+++	-	-	-
22	L. monocytogenes 2	+++	-	-	-
29	L. monocytogenes 3a	+++	-	-	-
30	L. monocytogenes 4a	+++	-	-	-
42	L. grayi	-	-	-	-

+++ strong agglutination reaction.

\- no reaction.

DISCUSSION.

DISCUSSION.

The results of the numerical taxonomic study (Figures 2 to 6 and Appendix III Figures I to IV) indicate a clear division of the strains into two distinct groups. The first of these two groups (cluster I) contains what can loosely be termed the 'lactic group'; representatives of the genera Erysipelothrix, Lactobacillus, Listeria and Streptococcus, together with the species Bacterium eurydice, Gemella haemolysans and Microbacterium thermosphactum. Also included in this group are the representatives of the genus Propionibacterium. The second major group (cluster II) includes the remaining members of the family Corynebacteriaceae - the genera Cellulomonas and Corynebacterium, the representatives of the family Brevibacteriaceae - genera Kurthia and Brevibacterium (except B. divaricatum), together with the representatives of the family Micrococcaceae - genera Staphylococcus and Micrococcus and the two possible Arthrobacter strains from throat swabs.

Cluster II.

The bacteria in cluster II will be discussed first because they were included in the survey as marker organisms and only single strains of the various species (except Kurthia) were included. As a result, the clustering within cluster II is relatively unstructured and very few conclusions can be drawn. It is obvious, however, that Kurthia zopfii is a tight (85% S), homogeneous species which fails to exhibit any close relationship to M. thermosphactum. This result supports the

views of Gardner (1969), who indicated that the only important characteristic shared by K. zopfii and M. thermosphactum (other than a morphological similarity), was that they were both frequently isolated from stored meat.

The members of the genus Staphylococcus, received as S. aureus, S. aureus (oxford strain), S. saprophyticus, S. epidermidis and Micrococcus hyicus, together with the one strain of Corynebacterium insidiosum, form a fairly tight cluster (83% S), IIB17. These results give added support to the view that M. hyicus is in fact a Staphylococcus (Jones, Deibel and Niven (1963)), but do not fully substantiate the suggestion by these authors that S. saprophyticus is probably synonymous with S. epidermidis. However, only one strain of each of these two species were included and there is some doubt about the identity of S. saprophyticus G175 (NCTC 7291) (see Jones, Deibel and Niven (1963)).

The strains of Cellulomonas, Corynebacterium and Brevibacterium clustered very loosely, and showed no obvious structure, but as previously mentioned, these genera were only included as marker organisms because of the present position of Listeria in the family Corynebacteriaceae (Bergey's Manual (1957)).

Cluster I.

Within cluster I the strains received as members of the genus Listeria, plus one received as E. rhusiopathiae, form a tight cluster at 83% S (cluster IA in Figure 2). Within this cluster three smaller clusters and one single strain can be distinguished:-

Cluster IA1 - 35 strains of L.monocytogenes and Listeria sp.,
1 strain received as L.denitrificans,
1 strain received as E.rhusiopathiae.

Cluster IA2 - 4 strains of L.grayi,
3 strains of L.murrayi.

Cluster IA3 - 3 atypical strains of L.monocytogenes and Listeria sp. (G8, G15a, G16a).

Strain G43 - L.denitrificans.

The species L. monocytogenes, as represented by cluster IA1, appears very homogeneous and clearly distinct from the representatives of the other three species and the three atypical strains (cluster IA3). The one strain received as E. rhusiopathiae, which falls in this cluster, was obviously wrongly identified, and should be renamed L. monocytogenes. It is unfortunate that more strains of L. denitrificans, L. grayi and L. murrayi could not be obtained, but those strains which were included give a good indication of the relationships within the genus. The representatives of L. grayi and L. murrayi form a tight cluster at 90% S (cluster IA2), and then join the L. monocytogenes cluster (IA1) at 85% S, which indicates a closer similarity to each other than to L. monocytogenes. Of the two strains of L. denitrificans, one (G1) clearly falls within the main L. monocytogenes cluster (IA1) and should be renamed L. monocytogenes, the other (G43) is strain L26 (Sohier, Benazet and Piechaud (1948)) upon which the species description is based, and appears quite distinct from the other three species of Listeria, although it shows a greater similarity to L. grayi and L. murrayi than to L. monocytogenes. However, when L. denitrificans (G43) was

included in a numerical taxonomic survey which contained a different set of 'coryneform' and 'lactic' bacteria, and in which a different set of tests were employed, it did not demonstrate such a close relationship to the other Listeria species (Jones pers.comm.), and infact clustered with some 'coryneform'strains. Its nearest neighbours (80% S) were 4 strains isolated from poultry deep litter by Schefferle (1966), tentatively designated Corynebacterium spp. Unfortunately, Schefferle listed insufficient characters to permit meaningful comparison of these strains with L. denitrificans.

The final cluster, IA3, contains the three 'atypical' strains which show less than 85% S to the other Listeria strains.

L. monocytogenes, therefore, appears to be a good species, but the position of L. grayi and L. murrayi is less clear. The representatives of these two latter species demonstrate at least 90% S to each other, the inclusion of more strains might have influenced the groupings, but such a high level of similarity does not appear consistent with the separation of the strains into two individual species.

A comparison of the results obtained in this study, with the reported characteristics of the four species of Listeria (see TABLE 1), can be seen in Table 29. there is good agreement between them. The characteristics of the four Listeria species are also compared with those of the 'atypical' Listeria of cluster IA3, and those reported for the 'atypical' lactobacilli from chicken meat, TS III (Thornley and Sharpe (1959), in TABLE 30. Characters which facilitate the separation of the four species of Listeria, cluster IA3 and members of TS

TABLE 29. Comparison of some reported characters of the four species of Listeria (a) with those obtained in the present study (b). (see TABLE 1 for references).

Characters	L.monocytogenes		L. grayi		L. murrayi		L.denitrificans	
	a	b	a	b	a	b	a	b
Anton eye test	+	.	+	.	-	.	-	.
Catalase	+	+	+	+	+	+	+	+
Motility	+	35+	+	+	+	+	+	+
-haemolysis	+	+	+	-	-	-	-	-
Voges-Proskauer test	+	+	+	+	+	+	-	-
Methyl red test	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	+	+	+	+
Aesculin hydrolysis	+	+	+	+	+	+	+	+
Acid production from-								
adonitol	-	-	-	-	-	-	-	-
L-arabinose	-	-	-	-	-	-	+	+
cellobiose	+	+	+	+	+	+	+	+
dextrin	+	.	+	.	+	.	+	.
dulcitol	-	-	-	-	-	-	-	-
erythritol	-	.	-	.	-	.	-	.
D-galactose	V/D	-	+	+	+	+	+	+
glucose	+	+	+	+	+	+	+	+
glycogen	-	.	-	.	-	.	-	.
inositol	-	-	-	-	-	-	-	-
inulin	-	-	-	-	-	-	-	-
lactose	V/D	33+	+	+	+	+	+	+

TABLE 29. (continued).

Characters	L. monocytogenes		L. grayi		L. murrayi		L. denitrificans	
	a	b	a	b	a	b	a	b
Acid production from-								
laevulose	+	+	+	+	+	+	+	+
maltose	+	+	+	+	+	+	+	+
mannitol	-	3+	+	+	+	+	-	-
mannose	+	.	+	.	+	.	+	.
melibiose	-	-	-	-	-	-	+D	-
melezitose	V	21+	-	(+)	-	+	-	+
raffinose	-	-	-	-	-	-	-	-
rhamnose	+	* +	-	-	+o	+ø	-	-
salicin	+	+	+	+	+	+	+	+
starch	+	.	+	.	+	.	+	.
sucrose	V/D	10+	-	-	-	-	+	-
trehalose	+	34+	+	+	+	+	+D	+D
xylose	-	11+	-	+	-	+	-	+
No. of strains-		37		4		3		1

\+ positive reaction (for sugars, within 48 hr.).

(+) weak positive reaction.

V variable.

. not tested.

D doubtful.

* rare strains rhamnose positive- 2/37 in present study.

o 6/9 strains positive.

ø 2/3 strains positive; 1/3 strains weakly positive.

34+ etc., actual no. of strains giving that reaction, otherwise all strains reacted in the same way.

TABLE 30. Comparison of the characters of the four species of Listeria with those obtained for cluster IA3 and those reported for TS III (Thornley and Sharpe (1959)).

Characters	L.monocytogenes	L.grayi	L.murrayi	L.denitrificans	IA3	TS III
[1]Anton eye test	+	+	-	-	.	.
Catalase	+	+	+	+	-	-
Motility	+	+	+	+	-	+
[1] -haemolysis	+	+	-	-	-	.
Voges-Proskauer test	+	+	+	-	+	.
Methyl red test	+	+	+	+	+	.
Nitrate reduction	-	-	+	+	-	-
Aesculin hydrolysis	+	+	+	+	+	+
Arginine hydrolysis	-	-	-	-	+	+
Acid production from-						
adonitol	-	-	-	-	1(+)	.
L-arabinose	-	-	-	+	-	-
cellobiose	+	+	+	+	+	+
[1]dextrin	+	+	+	+	.	+[s]
dulcitol	-	-	-	-	-/(+)/+	.
[1]erythritol	-	-	-	-	.	.
D-galactose	-	+	+	+	+	+
glucose	+	+	+	+	+	+
[1]glycogen	-	-	-	-	.	.
inositol	-	-	-	-	+	-
inulin	-	-	-	-	+	7+
lactose	33+	+	+	+	+	+[s]

TABLE 30. (continued).

Characters	L.monocytogenes	L.grayi	L.murrayi	L.denitrificans	IA3	TS III
Acid production from						
laevulose	+	+	+	+	+	+
maltose	+	+	+	+	+	+
mannitol	-	+	+	-	+	-
[1]mannose	+	+	+	+	.	+
melibiose	-	-	-	-	-/(+)/(+)	-
[1]melezitose	V	-	-	-	+	-
raffinose	-	-	-	-	-/(+)/+	-
[1]rhamnose	+*	-	+[o]	-	-/-/+	-
salicin	+	+	+	+	+	+
[1]starch	+	+	+	+	.	.
[1]sucrose	V/D	-	-	+	+	+
trehalose	+34	+	+	+D	+	+
xylose	a	a	a	a	-/(+)/(+)	-
no. of strains	37	4	3	1	3	8

\+ positive reaction (for sugars within 48 hr.)

(+) weak positive reaction.

. not tested / no reaction given.

V variable D doubtful

* rare strains are rhamnose positive

o 6/9 strains positive

a see previous table, the two sets of results do not agree.

1 result taken from TABLE 1, otherwise from present study.

34+ etc., no. of strains giving + reaction, otherwise all strains reacted in the same way.

III are given in TABLE 31 .

Members of the cluster IA3 resemble L. monocytogenes in their lack of nitrate reduction and production of acid from sucrose, and L. grayi and L. murrayi in their mannitol fermentation. They do, however, differ in several respects from the accepted Listeria groupings, they are non-motile, catalase negative, produce acid from inulin and inositol, and one strain (G15a) produces ammonia from arginine.

The characteristics of the TS III bacteria (Thornley and Sharpe (1959)) are shown in the final column of TABLE 30 , and except for the lack of demonstrable catalase, the production of acid from inulin and the hydrolysis of arginine, these bacteria resemble L. monocytogenes. They differ from L. grayi in their lack of catalase, arginine hydrolysis, acid production from inulin and failure to ferment mannitol. On comparison with cluster IA3, the TS III bacteria differ in their motility and failure to produce acid from inositol, mannitol or melezitose.

The characteristics of the TS II bacteria (Thornley and Sharpe (1959)) were also compared with those of Listeria. They are catalase negative, reduce litmus milk and hydrolyse arginine and aesculin, but are non-motile. These characters closely resemble those of the three bacteria of cluster IA3 in the present study. Thornley and Sharpe found that all of the strains within their group 2 (TSII) produced acid from cellobiose, dextrin, fructose, maltose, mannose, salicin, sucrose and trehalose, and failed to acidify arabinose, rhamnose, sorbose or xylose. These results are identical to those obtained for the Thornley and Sharpe Group 3 bacteria and all

TABLE 31. Characters which differentiate between the four species of Listeria, cluster IA3 and TS III, based upon Thornley and Sharpe (1959)[1], Welshimer and Meredith (1971)[2], and the present study [3].

Characters		L.monocytogenes	L.grayi	L.murrayi	L.denitrificans	IA3	TS[1] III
Anton eye test	[2]	+	+	-	-	.	.
Catalase	[2,3]	+	+	+	+	-	-
Motility	[2]	+	+	+	+	-	+
Voges-Proskauer	[2,3]	+	+	+	-	+	.
Nitrate reduction	[2,3]	-	-	+	+	-	-
Arginine hydrolysis	[3]	-	-	-	-	+	+
Acid from -							
L-arabinose	[2,3]	-	-	-	+	-	-
glycogen	[2]	-	-	-	+	.	.
inositol	[3]	-	-	-	-	+	-
inulin	[3]	-	-	-	-	+	7+
mannitol	[2,3]	34-	+	+	-	+	-
rhamnose	[2,3]	+*	-	+[o]	-	-/-/+	-
Reduction of Litmus Milk	[3]	+	+	+	-	+	+
RNase activity	[3]	+	+	+	-	+	.
Hippurate hydrolysis	[3]	+	-	+	+	+	.
Tween 60 "	[3]	34+	-	-	-	-	.
Cellulose "	[3]	-	-	-	+	-	.
No. of strains		37	4	3	1	3	8

Symbols - as in previous TABLE.

Listeria. With a further nine sugars and alcohols, however, ten different fermentation patterns could be distinguished within the group TS II. These patterns, together with the results given by TS III, cluster IA3 and the four species of Listeria are detailed in TABLE 32 . Some of the fermentation results resemble L. monocytogenes, eg., those of groups A,C,D and H; of these C also shows a resemblance to L.denitrificans, L. grayi and L. murrayi, while H shows an equal similarity to the four species of Listeria and the members of cluster IA3. Groups B, F, G, I and J, however, show a much greater similarity to the bacteria of cluster IA3. It is possible, therefore that these bacteria are also related to the genus Listeria, but perhaps insufficiently closely to justify placing them in the genus Listeria itself.

The IA3, TS II and TS III bacteria are among many lactic acid bacteria which have not, as yet been allocated to an exact taxonomic position. With the increased investigation of meat products held at low temperatures in conditions of reduced oxygen tension, and of sources such as animal house litter, it is possible that even more of these organisms may be isolated, Obviously this is an ideal area for further research and one that might prove very informative.

To return to the genus Listeria proper, it appears that L. monocytogenes is a well defined species, clearly distinct from L. grayi and L. murrayi, and that these two latter species probably do not differ sufficiently from each other to warrant individual specific status, L. murrayi merely being a biotype of L. grayi. L. grayi was named before L. murrayi (Larsen and Seeliger (1966)), and would,therefore,

TABLE 32. Comparison of the ten TSII groups (Thornley and Sharpe (1959) with the three bacteria of Cluster IA3 and the three 'species' of Listeria- L. monocytogenes, L. denitrificans and L. grayi / murrayi.

Characters.	BACTERIA. TS II.°										Cluster IA3.			L.monocytogenes	L.grayi/L.murrayi	L.denitrificans
	A	B	C	D	E	F	G	H	I	J	8*	15a*	16a*			
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Catalase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Reduction of Litmus Milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine hydrolysis	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-
Acid production from-																
galactose	-	+	+	-	-	-	-	+	-	+	+	+	+	V	+	+
lactose	-	+	+	-	-	-	+	+	+	+	+	+	+	V	+	+
melezitose	+	+	-	+	+	+	-	+	+	+	+	+	+	V	-(+)	-
raffinose	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-
inulin	-	+	-	-	-	+	+	-	+	-	+	+	+	-	-	-
glycerol	+	+	+	-	-	+	+	+	+	-	+	+	+	V	+	+
mannitol	-	+	-	-	-	+	+	+	+	+	+	+	+	-	+	-
inositol	-	-	-	-	-	+	+	-	+	-	+	+	+	-	-	-

\+ positive reaction. - negative result. V variable reaction.

* based upon TABLE 30.

° taken from Thornley and Sharpe (1959).

have priority over the more recent name L. murrayi (Welshimer and Meredith(1971)). All three of these species have a DNA base composition of between 37 and 40 moles % GC (Stuart and Welshimer (1973)), and although this does not prove relatedness, at least it does not prove dissimilarity!

L. denitrificans differs from L. monocytogenes and L. grayi/murrayi in many characters (see TABLE 33.).

TABLE 33. Characters which differentiate L. denitrificans from the other three species of Listeria, based upon Welshimer and Meredith (1971)[1], Stuart and Welshimer (1973)[2] and the present study[3].

Characters		L.monocytogenes, L.grayi and L.murrayi.	L.denitrificans.
DNA base ratio (moles%GC)	[2]	38±1	56±1
Voges-Proskauer test	[1,3]	+	-
Acid from L-arabinose	[1,3]	-	+
" glycogen	[1]	-	+
Reduction of Litmus Milk	[3]	+	-
RNase activity	[3]	-	+
Cellulose hydrolysis	[3]	-	+

\+ positive reaction.

\- negative reaction.

The numerical taxonomic work of Jones (pers. comm.) and the present study indicate that L. denitrificans is not a true member of the genus Listeria, and its DNA base composition of 56 moles% GC (see TABLE33) supports these conclusions.

The results of the serological study support those of the numerical taxonomic study. L. monocytogenes appears to be serologically distinct from L. denitrificans, L. grayi, L. murrayi and all the other bacteria tested, both for the 'O' and the 'H' antigens. L. denitrificans showed no antigenic similarity to any of the other species of Listeria, but L. grayi and L. murrayi appeared to be antigenically related.

Within the species L. monocytogenes, serotypes 1a (G32) and 2 (G22) showed a specific relationship to each other. This is consistent with their antigenic patterns (see TABLE 34), as they possess three common 'O' antigens. With the antiserum against serotype 1a (G32) both antigens produced three identical lines when tested by double gel diffusion. These presumably correspond to the three common antigens. With the antiserum against serotype 2 (G22) a single line of identity was produced, which can be assumed to correspond to one of these antigens. The antiserum against serotype 4a (G30) did not produce any reactions by double gel diffusion, and reacted non-specifically with all antigens by the ring precipitin test. It was not, therefore, possible to draw any conclusions from these results. The 3a antiserum (G29) reacted with 'O' antigens of serotypes 1a and 2 by the ring precipitin test, but only reacted with the homologous antigen by the double gel diffusion. These three serotypes (1a, 2 and 3a) do possess two common somatic antigens, and the results of the ring

TABLE 34. Antigenic patterns of Listeria monocytogenes.

SEROTYPE:	'O' FACTORS:	'H' FACTORS:
1a	I, II, (III)*	A, B
1b	"	A, B, C
2	"	B, D
3a	II, (III), IV	A, B
3b	"	A, B, C
4a	(III), (V), VII, IX	"
4b	(III), V, VI	"
4c	(III), V, VII	"
4ab	(III), V, VI, VII, IX	"
4d	(III), VI, VIII	"
4e	(III), V, VI, VIII, IX	"

* Parentheses indicate irregularly occuring factors.

precipitin test are compatible with this.

The four serotypes have at least one 'H' antigen in common with each other (see TABLE 34). The absorbed 'H' antisera against serotypes 1a and 2 reacted with all the L. monocytogenes 'H' antigens (serotypes 1a, 2, 3a, 4a plus G 13,

G 14, G 31 and G 35). The absorbed 'H' antisera against serotypes 3a and 4a reacted similarly, except that no reactions were observed with the type 1a and 2 'H' antigens (see TABLE 26). The absence of B factor antigen or antibody would explain the lack of reaction with serotype 2 antigen, but the absence of any agglutination with the 1a antigen is not so easily explained.

The L. murrayi 'O' antiserum was specific, only reacting with the homologous HCl extract and that of C. poinsettiae G158 (this latter bacterium reacted with all the 'O' antisera against which it was tested). The L. grayi 'O' antiserum, in contrast, was completely non-specific and reacted with all of the HCl extracts with which it was tested. The L. grayi 'H' antiserum, however, was specific (see TABLE 26). After absorption with L. grayi 'O' antigens, the 'H' antiserum (diluted 1 in 1 with saline) reacted with L. grayi and L. murrayi 'H' antigens to a titre of 1 : 30,768. In comparison, extremely low titres ($\leq$1 : 32) were obtained with the L. monocytogenes 'H' antigens. Although attempts were made to prepare L. murrayi 'H' antiserum, no success was achieved, which may be due to a variety of reasons - poor development of flagellae, failure of the rabbit to react to the antigens etc. It would have been interesting to perform the reciprocal tests, but the results do demonstrate the possession of at least one antigen in common. Welshimer and Meredith (1971) reported that L. grayi and L. murrayi possess 'H' antigens in common with each other, but not with L. monocytogenes (see TABLES 35 & 36). They also reported a complex mixture of 'O' antigens shared and unshared by all the L. grayi and L. murrayi strains

TABLE 35. Agglutination tests with different 'O' and 'H' antigen suspensions, and antisera to two strains of L.murrayi (ATCC 25401 (G44) and 25403 (G46)), based upon Welshimer and Meredith (1971).

Test Antigens	Titre Obtained	
	ATCC 25401	ATCC 25403
a) 'H' antigens	'H' antisera*	
L. monocytogenes		
serotype 1	0	0
" 2	0	0
" 4b	0	0
L. grayi	1,280	20,480
ATCC 25400	10,240	20,480
L. murrayi		
ATCC 25401	10,240	10,240
ATCC 25402	10,240	5,120
ATCC 25403	10,240	10,240
b) 'O' antigens	'O' antisera	
L. monocytogenes		
serotype 1	0	0
" 2	320	320
" 4a	20	20
" 4b	0	0
L. grayi	1,280	1,280
ATCC 25400	1,280	2,560
L. murrayi		
ATCC 25401	1,280	1,289
ATCC 25402	1,280	2,560
ATCC 25403	1,280	2,560

* These antisera have been absorbed with their homologous 'O' antigens prior to performing the tests.

TABLE 36. 'H' antigen patterns of L. grayi (ATCC 19120 and 25400 (G47)) and L. murrayi (ATCC 25401 (G44), 25402 (G45) and 25403 (G46)), based on Welshimer and Meredith (1971).

'H' Antigens	L.grayi ATCC 19120	L.grayi (G47) ATCC 25400	L.murrayi (all 3 strains)
F	+	+	+
G	+	+	
H		+	+

F, G and H - designation of Welshimer and Meredith (1971).

tested. 'O' antisera against two strains of L. murrayi reacted with L. monocytogenes serotypes 3 and 4a at low dilutions (see TABLE 35). These low level reactions with L. monocytogenes are compatible with results obtained in the present study - when L. grayi and L. murrayi were tested against L. monocytogenes 'H' antisera low level reactions were obtained which were removed when the antiserum was absorbed with the homologous 'O' antigens, suggesting some very low level of antigenic similarity.

L. denitrificans showed no 'O' antigenic similarity to any of the other strains of Listeria, nor did it react with any of the absorbed 'H' antisera produced against them. Unfortunately, it proved impossible to prepare any 'H' antisera against L. denitrificans; studies with such antisera may possibly have helped to clarify the position. Welshimer and Meredith (1971) also found a complete lack of common antigens between L. denitrificans and the other species of Listeria, supporting the findings of the present study and the earlier suggestion (page 146) that L. denitrificans is not a true member of the genus Listeria.

The strain G1 received as L. denitrificans, which fell within the L. monocytogenes cluster (IA1) in the numerical taxonomic survey, showed no 'O' antigens in common with L. denitrificans, L. murrayi or L. grayi, but did react with L. monocytogenes serotype 3a antisera. It was also tested against the five Listeria 'H' antisera (after absorption with their homologous 'O' antigens) by slide agglutination. No reaction was observed with the L. grayi 'H' antiserum, but the cells were agglutinated by all four L. monocytogenes 'H' antisera.

These results all support the renaming of strain G1 as L. monocytogenes, not L. denitrificans.

For reasons stated earlier, HCl extracts of a representative of each of the three groups of bacteria described by Thornley and Sharpe (1959) were tested against all the Listeria 'O' antigens, no positive reactions were produced. Also, the representative of TS III was tested by slide agglutination against all of the Listeria 'H' antisera (after they had been absorbed with their homologous 'O' antigens), and again no positive reactions were observed. These results support the conclusions from the discussion of the numerical taxonomic study, that these bacteria show some similarity to the Listeria group, but do not demonstrate sufficient similarity to be placed within the actual genus Listeria at this time.

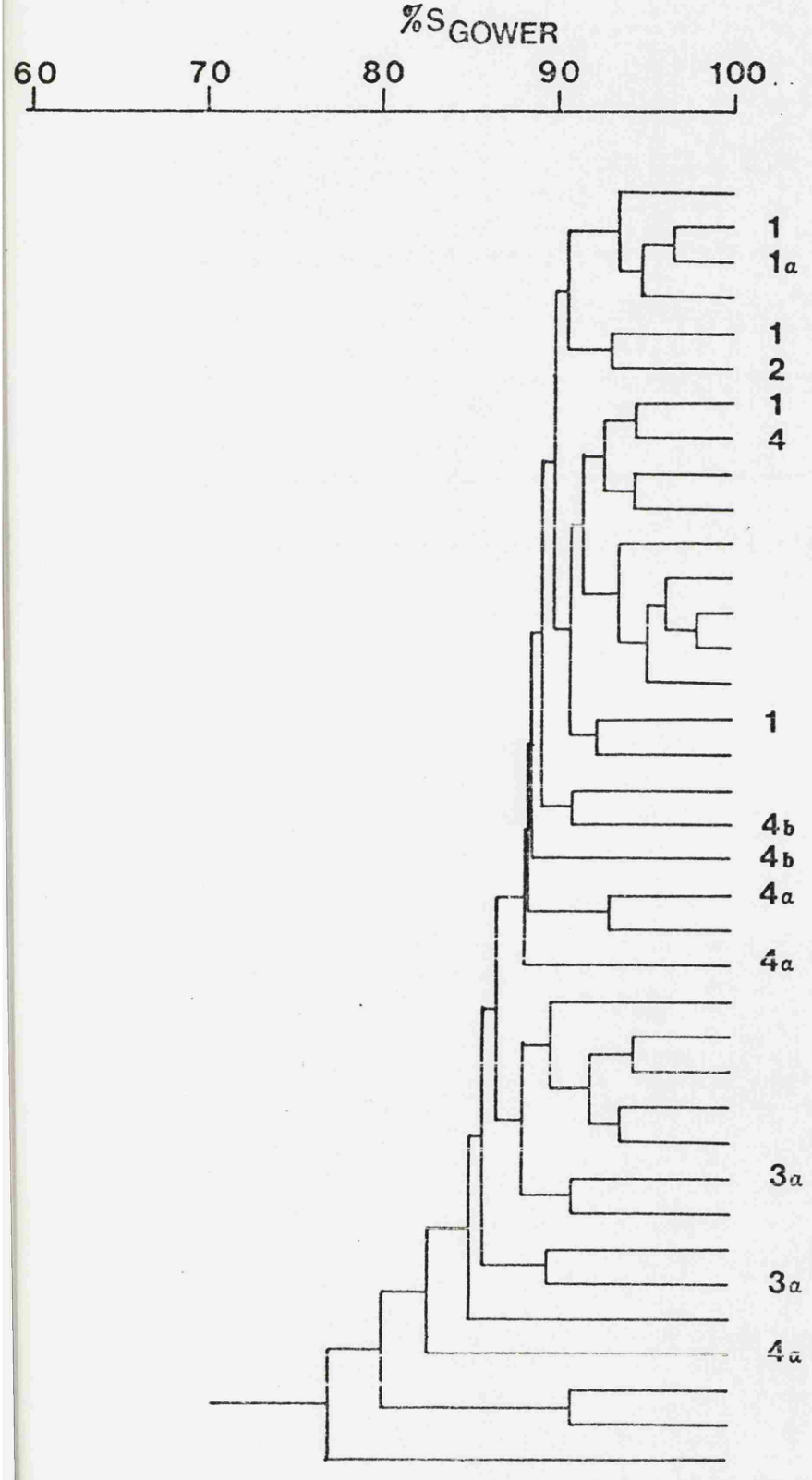
The serotypes of 14 of the strains of L. monocytogenes (cluster IA1) were known (see TABLE 37). Their distribution in the various dendrograms produced in the numerical taxonomic study was investigated, in order to ascertain whether there was a relationship between the serological types of L. monocytogenes and the physiological groupings (see Figure 13). No such relationship could be found.

HCl extracts of the 37 strains in cluster IA3 (Figure 2) were tested with the antisera produced against the four Listeria serotypes in the present study. The results (see TABLE 24) did not indicate any relationship between serological types and numerical taxonomic clusters. The results did, however, indicate that some strains were more antigenically active, some reacting strongly with all four 'O' antisera, others reacting weakly with one or two, and others failing to react at all.

TABLE 37. Serological types of the 14 strains of L. monocyogenes of which the serotype was known.

Strain No.	Serological Types.						
	1	1a	2	3a	4	4a	4b
G 5	+						
G 6							+
G 7					+		
G 10						+	
G 11						+	
G 12	+						
G 13	+						
G 14	+						
G 17							+
G 22			+				
G 24				+			
G 29				+			
G 30						+	
G 32		+					

Figure 13 . Dendrogram of the L. monocytogenes cluster (IA1) as obtained in the present numerical taxonomic study (see Figure 2.) showing the serological types where known.



All the evidence that we were able to obtain, therefore, indicates that serotypes within the species L. monocytogenes are not linked to physiological types. These results are supported by the work of Stuart and Pease (1972) who also found no correlation.

It appears that cluster IA is distinct from clusters IB and IC (see Figure 2), but the division between these two latter clusters may be artificial (see Sneath and Johnson (1972)). Within the IB-IC grouping, however, there are 14 clusters obviously warranting taxonomic rank. The art of taxonomy is a subjective procedure and all the groups within clusters IB and IC are worthy of individual discussion.

Erysipelothrix rhusiopathiae (cluster IC14) appears to be a distinct, homogeneous species; the overall similarity of the strains is 90% and no clear subdivisions can be observed within the cluster. No close taxonomic relationship to Listeria is indicated, the similarity between these two genera being no greater than that shown by either of them to the various members of the Lactobacillaceae or to M. thermosphactum. These results, together with the preliminary findings of Stuart and Pease (1972), indicate that Listeria and Erysipelothrix are two separate genera, possibly within the same family, not two species of the same genus.

Erysipelothrix demonstrates a reasonably close relationship (78-80% S) to the bacteria at present referred to as Gemella haemolysans and Bacterium eurydice, as well as to the streptococci of serological groups F, K, M and O and S. suis (clusters IC10 and IC11). Its similarity to the other members of the family Lactobacillaceae, Listeria and Microbacterium

thermosphactum is at least 72% S, whereas the group shows a much lower similarity to the members of the family Corynebacteriaceae included in cluster II. Clearly these results support the views of Davis, Fomin, Wilson and Newton (1969) and Stuart and Pease (1972) who suggested that Erysipelothrix was more closely related to the Lactbacillaceae than the Corynebacteriaceae. The fact that Erysipelothrix produces lactic acid, is catalase and cytochrome negative, lacks "coryneform" morphology and has a DNA base ratio of 38 to 40 moles% GC (Flossmann and Erler (1972)) indicates that its correct taxonomic position is in the Lactobacillaceae, and that is where the present survey has placed it!

Microbacterium thermosphactum formed a tight cluster (IB12) at 90% S in which no clear internal structure was observed, indicating that M. thermosphactum is a tight, homogeneous species. Its closest similarity was to the third Lactobacillus cluster (IB6- which contained representatives of the species L. casei, L. plantarum and L. salivarius) and to the distinct cluster formed by strains of L. mali (IB7). M. thermosphactum exhibited a much closer relationship to the members of the family Lactobacillaceae, Listeria and E. rhusiopathiae than it did to the family Corynebacteriaceae. Many characteristics of M. thermosphactum closely resemble those of the Lactobacillaceae. Its DNA base ratio of 36 moles % GC is very similar to that of the streptococci and some of the lactobacilli (see TABLE 39), it produces lactic acid (McLean and Sulzbacher (1953) and groups with the members of the Lactobacillaceae both in this study and in a study by Davis, Fomin, Wilson and Newton (1969).

The main characteristics which have always been quoted as justification for not placing M. thermosphactum in the Lactobacillaceae are its production of catalase and cytochromes. It has been shown, however, that some members of the family Lactobacillaceae do produce catalase, both the so-called pseudocatalase and the classical catalase which contains haem (Dacre and Sharpe (1956), Vaňková (1957) and Whittenbury (1964). It does not, therefore, seem justifiable to exclude M. thermosphactum from the Lactobacillaceae on the basis of this character.

Lactobacillus strains formed four separate clusters in the present study, of these, one (IB7) contained representatives of the species L. mali, while the other three corresponded closely to the three subgenera proposed by Orla-Jensen (1919). The groupings obtained in the present study, those of Orla-Jensen (1919) and the extended version produced by Rogosa and Sharpe (1959) are compared in TABLE 38 .

The main differences between the groupings obtained in the present study and those generally accepted, are the positions of L. leichmannii, L. salivarius var salivarius and L. salivarius var salicinius. The two sub-species of L. salivarius were placed in the subgenus Thermobacterium by Rogosa and Sharpe (1959), but fall in the subgenus Streptobacterium in the present study, while the homofermentative L. leichmannii grouped in the heterofermentative subgenus Thermobacterium. It is also interesting to note that L. acidophilus biotype I (G61) is attached at a fairly low level of similarity (76% S) to clusters IC4 and IC5 and does not show a close relationship to L. acidophilus (G60) in the

TABLE 38. Comparison of the groupings within the genus Lactobacillus, as recognised by Orla-Jensen (1919) and extended by Rogosa and Sharpe (1959) with those formed in the present study.

Orla-Jensen	Rogosa and Sharpe	Present study
a) Thermobacterium.		
T.lactis	L.lactis	L.lactis
T.bulgaricum	L.bulgaricus	L.bulgaricus
T.helveticum	L.helveticus	L.helveticus
T.jogurt	L.jogurti	L.jugurt
	L.acidophilus	L.acidophilus
	L.delbrueckii	L.delbrueckii
	L.leichmannii	
	L.salivarius var salivarius	
	L.salivarius var salicinius	
T.cereale		
b) Betabacterium		
B.breve	L.brevis	L.brevis
B.fermentum	L.fermenti	L.fermentum
B.longum		
B.caucasicum		
	L.buchneri	L.buchneri
	L.cellobiosus	L.cellobiosus
	L.viridescens	
		L.leichmannii*

TABLE 38 (continued).

Orla-Jensen	Rogosa and Sharpe	Present study
c) Streptobacterium		
S.casei	L.casei	L.casei
		L.casei subsp. rhamnosus
S.plantarum	L.plantarum	L.plantarum
		L.salivarius var.* salivarius
		L.salivarius var.* salicinius

* strains which did not show the accepted grouping in the present study.

present study. Clearly, since only one representative of most species was included, and tests were not selected specifically for the differentiation of the lactobacilli, unequivocal results were not expected for this group, they were included as markers. Considering the limitations, therefore, agreement between the sets of groupings was good.

It is interesting to note that the heterofermentative subgenus, Betabacterium, and the homofermentative subgenus, Thermobacterium, (clusters IC4 and IC5) showed a closer relationship to each other (80% S) than to the other homofermentative subgenus, Streptobacterium (IB6). This latter subgenus in fact, clustered in a different subgroup of cluster I (IB) from the other two subgenera (IC), and showed its closest similarity (77 - 79% S) to the L. mali cluster (IB7) and to M. thermosphactum (IB12). Since the similarity levels between clusters IB and IC, and between their component clusters were so close (Figure 2), little emphasis can be placed upon this separation of the Lactobacillus subgroups.

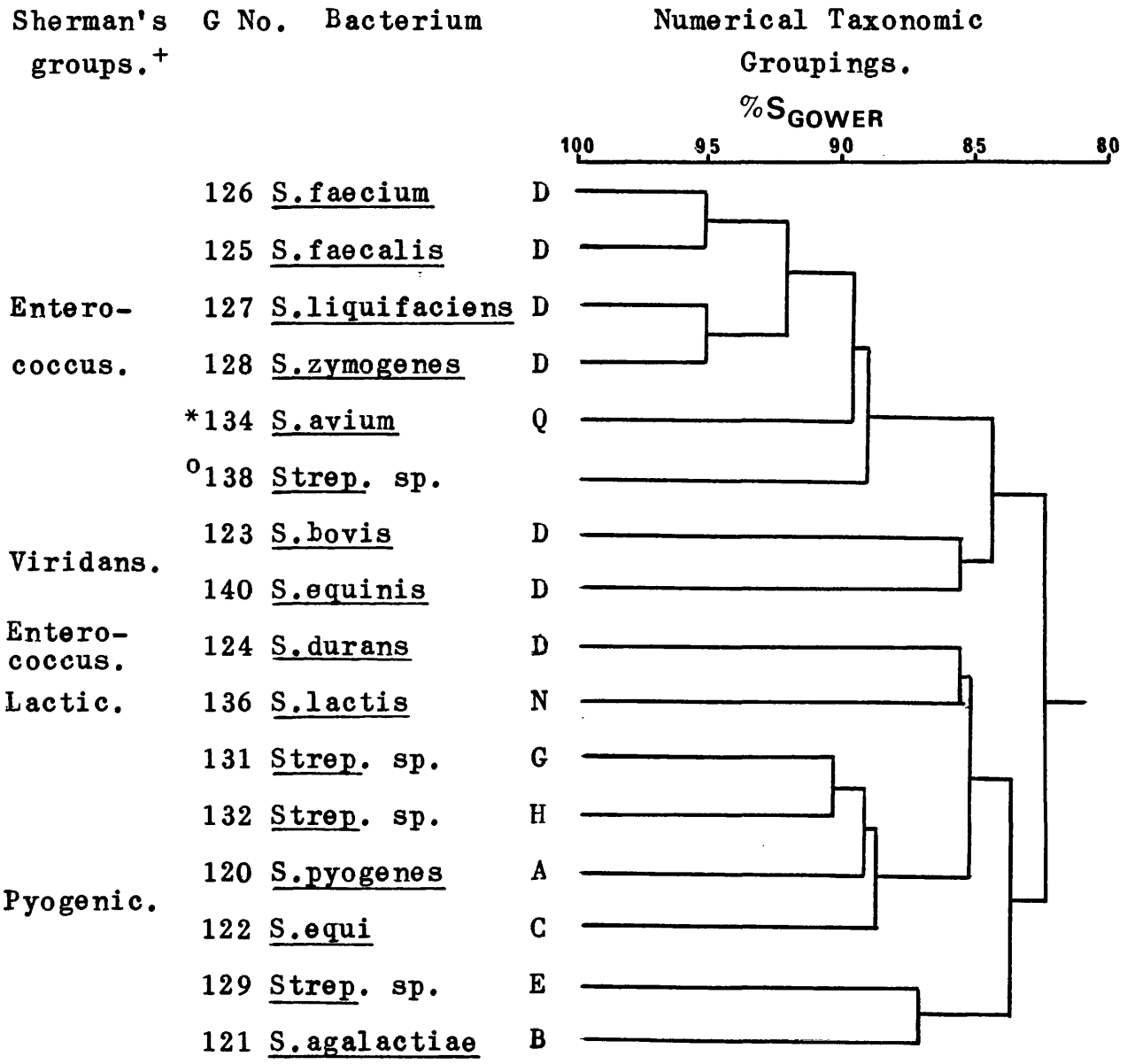
The present results, therefore, give a good correlation with the groupings of Orla-Jensen (1919) and the extended groupings of Rogosa and Sharpe (1959), and also indicate a close relationship between the lactobacilli and strains of B. eurydice, Erysipelothrix, Gemella haemolysans, M. thermosphactum and, of course, Streptococcus.

The position of the three strains of L. mali isolated by Carr and Davies (1970) from cider, is unclear. They form a tight cluster (95% S) and group in the region of the clusters of M. thermosphactum (IB12), Lactobacillus subgenus Streptobacterium (IB6) and Propionibacterium (IB16), their

classification in the genus Lactobacillus, therefore, is probably correct.

Streptococcus strains formed five clusters in the present study, one (IC9) contained 3 strains from serological groups F and K, another (IC10) contained one strain of S.suis (G141 - Elliot (1966), strain PM 1) and one strain each from serological groups O and M, while cluster IC11 contained the remaining 5 strains of S. suis. The other two clusters (IB8 and IB9) contained the majority of the Streptococcus strains. The internal arrangement of these two latter groups shows an interesting agreement with the groups of Sherman (1937); the groupings are compared with Sherman's divisions in Figure 14 . The strain of S. durans (G124), Sherman's 98D, the type strain, did not cluster with the other enterococci, but linked loosely to S. lactis (serological group N). In the study of Jones, Sackin and Sneath (1972) this strain clustered on the very edge of the S. durans group, perhaps one of their more central strains would have clustered more closely with the Group D streptococci in the present study. The other members of the enterococcus group form a tight cluster, joined by the two group D "viridans" species, S. bovis and S. equinis, and this cluster is then joined by the remaining streptococci within these clusters (representatives of the pyogenic and lactic groups, plus S. durans). The grouping of the pyogenic streptococci is reasonably good, but includes S. durans and the one lactic Streptococcus, S. lactis. In view of the fact that each species of Streptococcus was only represented by a single strain (except S. suis) the agreement which these results show with those of Sherman (1937),

Figure 14 . A Comparison of the groupings obtained in the present numerical taxonomic study with those of Sherman (1937).



+ Based upon Sharpe, Fryer and Smith (1966).

* This species was placed in the Enterococcus group by Nowlan and Deibel (1967).

o Serological group unknown.

Sharpe, Fryer and Smith (1966) and Jones, Sackin and Sneath (1972) is most notable.

Gemella haemolysans (3 strains) and the one strains received as Neisseria haemolysans, formed a tight cluster (IC15) at 92% S, which was clearly distinct from the other bacteria in the survey. It, therefore, appears that G. haemolysans is a distinct homogeneous species. Its closest relationship was to Erysipelothrix, Bacterium eurydice, Streptococcus cluster IC10 and S. suis, and it clearly fell within the 'lactic' area. Reyn (1969 and 1970) and Reyn, Birch-Andersen and Berger (1970) suggested that G. haemolysans should be reclassified in a family of Gram positive bacteria because its cell wall, internal structure and mode of division were all typical of Gram positive bacteria. They suggested that the family should probably be the Lactobacillaceae, in the tribe Streptococceae. Its DNA base ratio of 33.5 moles% GC is compatible with the 33.5 - 42 moles% GC reported for the genus Streptococcus (see TABLE 39), as are its coccal form and physiological and biochemical characteristics. The results of the present study, therefore, substantiate the suggestion by Reyn (1969 and 1970) and Reyn, Birch-Andersen and Berger (1970) that Gemella haemolysans should be transfered to the tribe Streptococceae, and its lack of catalase and cytochromes is also consistent with such a transfer.

Bacterium eurydice was represented by three strains which formed a tight cluster (IC19) at 93% S, distinct from the other groups within cluster IC. The group clearly fell within the 'lactic acid' area and showed 77 -81% similarity to G. haemolysans (IC15), Erysipelothrix (IC14), S. suis

(IC11) and one Streptococcus cluster (IC10). Classification in the family Lactobacillaceae could be proposed on the basis of this one set of results, but further substantiation of this relationship would be advisable before any recommendations were made.

Brevibacterium divaricatum is the only strain of this species to fall within the 'lactic group' (cluster I) in this survey, and it exhibited over 80% similarity to B. eurydice. It obviously shows a closer relationship to the family Lactobacillaceae than the families Brevibacteriaceae and Corynebacteriaceae, and it would be interesting to investigate its taxonomic position further. Little can be deduced on the basis of this single study, but perhaps one species of the genus Brevibacterium is a little closer to finding its correct taxonomic position.

Propionibacterium was represented by single strains of four of its constituent species. They formed a tight cluster (80% S) which fell within the 'lactic' group (cluster I) and exhibited a 73 - 75% similarity to the other members of the cluster, the lactobacilli, streptococci, Listeria, Erysipelothrix, M. thermosphactum and Gemella. This similarity was higher than that obtained by Seyfried (1968) of 62 - 63% similarity to the genera Lactobacillus and Streptococcus. However, Seyfried included only representatives of these three genera in her study, but it did appear that the three clusters were all of equivalent taxonomic status, not necessarily within the same family. Clearly, Propionibacterium, despite its pleomorphic nature, shows a greater resemblance to the 'lactic' group than to the family Corynebacteriaceae in the present

study, and little more can be deduced from an investigation in which the genus Propionibacterium was represented by just four strains.

The results of the serological study support those of the numerical taxonomic study.

The 'O' antiserum produced against E. rhusiopathiae (G190) reacted strongly by the ring precipitin test with the homogeneous HCl extract, and very weakly with that of G49 Streptococcus sp. (serological group P). This latter reaction, unlike the homologous reaction, could not be detected by double gel diffusion, and was probably a non-specific cross reaction.

The M. thermosphactum 'O' antiserum reacted strongly by the ring precipitin test with the homologous HCl extract, and produced a very faint ring with G135, Streptococcus sp. (serological group M) after 1 hour. This reaction appeared to be a weak, non-specific cross reaction comparable with that observed between Erysipelothrix and the G49 HCl extract.

The antiserum prepared against G. haemolysans ('O' antiserum) also reacted strongly in the ring precipitin test with the homologous HCl extract, and weakly with that of G135, Streptococcus sp. serological group M. By double gel diffusion the homologous reaction produced an intense white line, but no reaction was detected against the G135 HCl extract, which again suggested that a weak, non-specific cross reaction had occured.

Apart from a few, weak, probably non-specific, cross reactions, therefore, E. rhusiopathiae, G. haemolysans and M. thermosphactum were found to possess 'O' antigens serologically distinct from those of each other and those of all the other

strains which were tested.

The two strains of Lactobacillus which were tested, L. casei and L. bulgaricus, also appeared to be serologically distinct. L. bulgaricus (G64) 'O' antiserum reacted strongly with the homologous HCl extract and weakly with that of G49, Streptococcus sp. (serological group P), by the ring precipitin test. By double gel diffusion only the reaction with the homologous HCl extract was detected. The reaction with G49 was probably similar to the non-specific reaction observed between the E. rhusiopathiae 'O' antiserum and G49. L. casei 'O' antiserum reacted by the ring precipitin test with the homologous HCl extract and those of G132 (Streptococcus sp. group H), G168 (Kurthia zopfii), G158 (C. poinsettiae) and G64 (L. bulgaricus). By double gel diffusion three lines were observed with the homologous HCl extract, the fainter, more central line of which was shared with the other four HCl extracts (see Figure 11). There was insufficient time available to absorb this antiserum, but it did appear that except for a non-specific reaction with C. poinsettiae, K. zopfii, L. bulgaricus and Streptococcus sp. (group H), L. casei possessed distinct 'O' antigens.

The 'O' antiserum produced against K. zopfii not only reacted with the homologous HCl extract, using the ring precipitin test, but also with HCl extracts of L. monocytogenes type 4a (G30) and C. poinsettiae (G158). The results of the subsequent double gel diffusion tests were rather unexpected (see Figure 11). All three HCl extracts produced an identical faint line with the K. zopfii 'O' antiserum, the L. monocytogenes HCl extract, however, produced an intense line, and C.

poinsettiae a different, fainter line in addition to the first line. There was insufficient time available for these reactions to be investigated further by absorption, but the original test bleed (prior to injection) of the rabbit from which the antiserum was obtained, was tested for any reactions with L. monocytogenes serotype 4a (G30) and C. poinsettiae HCl extracts. No such reactions were found. These reactions could possibly indicate the presence of an antigen, or antigens, reported to be fairly common in Gram positive bacteria, e.g., the Rantz antigen (Neter, Anzai and Gorzynski (1960)) and the antigen reported by Stuart and Pease (1972).

The Streptococcus 'O' antisera, with the exception of those produced against serological groups B and L (G121 and G48), only reacted with their homologous HCl extracts and that of C. poinsettiae. The 'O' antiserum produced against S. agalactiae (G121) showed a very weak reaction with the representative of TS II, after 30 minutes. The antiserum against G48, a Streptococcus sp. of serological group L, produced weak reactions with C. poinsettiae (G158), K. zopfii (G168), L. bulgaricus (G64), Listeria grayi (G42), Listeria murrayi (G44) and M. thermosphactum (G104), by the ring precipitin test. These HCl extracts were then tested against the antiserum by double gel diffusion, lines were only observed for the homologous reaction, the weak reactions observed by the ring precipitin test were probably weak, non-specific reactions. Again, it was not possible to absorb the antisera, but the results clearly indicate that the streptococci are serologically distinct from all of the other bacteria included in the study.

Those bacteria in the study which were known to be motile, were tested by slide agglutination with the absorbed Listeria 'H' antisera (see TABLES 26 and 28). No flagella antigenic relationship could be detected between Listeria (L. monocytogenes serotypes 1a, 2, 3a and 4a and L. grayi) and C. poinsettiae, K. zopfii, Lactobacillus delbrueckii, S. faecalis (representing the streptococci - the majority of which were found to be motile in the present study) and TS III (shown to be motile by Thornley and Sharpe (1959) and Jones (pers. comm.)).

The serological results, therefore, indicate that E. rhusiopathiae, G. haemolysans, M. thermosphactum, the two species of Lactobacillus and the streptococci which were tested are all antigenically distinct, thus supporting the numerical taxonomic results.

C. poinsettiae HCl extracts exhibited a completely non-specific cross reaction with all the 'O' antisera tested in the present study (by the ring precipitin test). The reaction was not detected by double gel diffusion tests, except with the antisera produced against L. casei and K. zopfii. Further investigation of this cross reaction should be considered, as mentioned previously reactions such as these have been reported by Neter, Anzai and Gorzynski (1960) and Stuart and Pease (1972). There have been many reports in the literature (Seeliger (1961), Gray and Killinger (1966) that the serological reactions with Listeria can be quite non-specific. This is of practical importance with human sera from suspected clinical cases, which often react with streptocci, C. poinsettiae and all the Listeria serotypes. The results from the

present serological study indicate that it might be useful to absorb clinical sera with C. poinsettiae before testing with Listeria antigens, because C. poinsettiae HCl extracts never showed the specific lines in the double gel diffusion tests which the homologous Listeria HCl extracts produced.

In conclusion, the present study, together with previous investigations, indicates that L. monocytogenes is a homogeneous species, clearly distinct from L. grayi and L. murrayi which are probably varieties of the same species of Listeria. L. denitrificans differs to such a great extent from the other three species of Listeria that it should clearly be reclassified, but the results of the present study do not permit the suggestion of an alternative taxonomic position.

Listeria was shown to be distinct from Erysipelothrix, and the suggestion by Miles and Wilson (1957) that they should be placed in the same genus was not supported.

M. thermosphactum clearly does not resemble Kurthia, and the views of Gardner (1969) were therefore substantiated.

The divisions of the streptococci and lactobacilli in the present study show a good correlation with the Streptococcus groups of Sherman (1937) and the Lactobacillus subgenera of Orla-Jensen (1919).

The results also indicate that Listeria, Erysipelothrix and M. thermosphactum should be removed from the family Corynebacteriaceae. They more closely resemble the family Lactobacillaceae, and it is possible that the family could be extended to contain them, or, alternatively, a new family could be created for some or all of these displaced bacteria.

Erysipelothrix is catalase and cytochrome negative (see

TABLE 39) and would, therefore be acceptable in the family Lactobacillaceae as it is at present defined. It bears a closer resemblance to the tribe Streptococceae than to the tribe Lactobacilleae, but would be excluded from the former tribe on the basis of its rod-shaped morphology, and a new tribe would probably be required.

The genus Microbacterium, including M. thermosphactum, was at one time (see Introduction) a member of the family Lactobacillaceae. Lactic acid producing, Gram positive, rod-shaped bacteria were divided between the two genera, Lactobacillus and Microbacterium, on the basis of the catalase test. M. thermosphactum is catalase and cytochrome positive, and as such would be excluded from the family Lactobacillaceae as it is at present defined.

Listeria is catalase positive, but its status with regard to the presence of cytochromes is less clear. The detection of these compounds is highly dependent upon the nature of the medium upon which the bacteria are grown prior to testing (Smith (1954)). Keeler and Gray (1960) reported a low level of cytochromes, whereas Trivett and Meyer (1971) reported that Listeria is completely lacking in cytochromes. Jones (pers. comm.) has detected a low level of cytochrome activity associated with the cell membrane of Listeria.

There are increasing numbers of reports in the literature of the detection of catalase, or cytochromes, or both, in existing members of the family Lactobacillaceae (Dacre and Sharpe (1956), Vaňková (1957), Whittenbury (1964)). The new species, Lactobacillus mali, showed such a close resemblance to the other lactobacilli that Carr and Davis (1970)

TABLE 39. Comparison of some characters of possible members of the family Lactobacillaceae with those of Streptococcus and Lactobacillus.

GROUP	moles % GC	catalase activity	presence of cytochromes	rods/ cocci
Lactobacillus	39 - 51.2 [1,2]	-*	-*	rods
Streptococcus	33.5 - 42 [2,3]	-	-	cocci
Listeria	38 [4]	+	+o	rods
Erysipelothrix	38 [5]	-	-	rods
M. thermosphactum	.	+	+	rods
Gemella	33.5 [6]	-	-	cocci
B. eurydice	.	-	.	rods

* there have been reports that some strains are, in fact, positive (see text).

o there have been varying reports that cytochromes are present in low levels, or absent. It seems most probable that Listeria does in fact possess low levels of cytochrome, probably associated with the cell membrane (see text).

TABLE 39. (continued).

References:-

1 Cantoni, Hill and Silvestri (1965).

2 Marmur and Doty (1962).

3 Belozersky and Spirin (1960).

4 Stuart and Welshimer (1973).

5 Flossman and Erler (1972).

6 Berger (1961).

placed it in this genus despite its catalase activity. In view of such reports a good case can not be made for the exclusion of Listeria or M. thermosphactum from the family Lactobacillaceae on the basis of their catalase and cytochrome production.

Gemella haemolysans is catalase and cytochrome negative (Berger (1961)) and its resemblance to the tribe Streptococceae, reported by Reyn (1969 and 1970) and Reyn, Birch - Andersen and Berger (1970) has been substantiated by the present study. Its coccal morphology and DNA base composition (see TABLE 39) are clearly in keeping with such a taxonomic position.

Bacterium eurydice is also catalase negative, and has demonstrated a close resemblance to the family Lactobacillaceae in the present study, but its cytochrome content and DNA base ratio are not known. It is possible that B. eurydice should also be placed in the family Lactobacillaceae, but this move requires further substantiation.

The bacteria of cluster IA3 (present study), TS II and TS III (Thornley and Sharpe (1959)) are further possible members of this extended family Lactobacillaceae, but their taxonomic position is still very uncertain and warrants further investigation. A logical progression of the present study would be a study of these, at present unclassified bacteria, using markers from cluster I of the present study, as it is important that they should be allocated to a more accurate taxonomic position. TABLE 40 shows a possible membership of the new, enlarged family Lactobacillaceae which has been discussed here.

Table 40. A possible list of members for the enlarged family Lactobacillaceae, as proposed in the text.

FAMILY:	Lactobacillaceae
TRIBE I:	Lactobacilleae
GENERA:	Lactobacillus
	Eubacterium
	Catenabacterium
	Ramibacterium
	Cillobacterium
TRIBE II:	Streptococceae
GENERA:	Streptococcus
	Diplococcus
	Pediococcus
	Leuconostoc
	Peptostreptococcus
	+Gemella
TRIBE III:	
GENERA:	Erysipelothrix
	?Bacterium eurydice
TRIBE IV:	
	Microbacterium thermosphactum
	*Listeria

* Listeria could possibly be placed with M. thermosphactum, or it could be placed in a separate tribe, or even a separate Family.

A possible alternative to such a marked enlargement of the family Lactobacillaceae is the creation of a new family. The genus Listeria does not appear to be quite as closely related to the streptococci and lactobacilli as are Gemella, Erysipelothrix and M. thermosphactum. It could be argued that a new family, the Listeriaceae perhaps, should be created for members of the genus Listeria and the atypical Listeria of cluster IA3 (present study), and it might even prove a suitable home for at least some of the unclassified 'lactic acid' bacteria mentioned earlier.

Obviously it is wrong to create new families and tribes without true justification, but it is also wrong to lump together bacteria which do not exhibit a true affinity to each other. The final taxonomic position of Erysipelothrix, Gemella, Microbacterium thermosphactum, Listeria, Bacterium eurydice and the atypical lactobacilli and Listeria will have to be decided between the 'lumpers' who may wish to place them all in the family Lactobacillaceae, and the 'splitters' who may prefer the creation of a new family for some, or all, of them.

APPENDIX I.

STRAINS USED IN THE NUMERICAL TAXONOMIC SURVEY.

One hundred and ninety three strains were selected for study, these are listed in TABLE I. Type strains as indicated by Sneath and Skerman (1966), and recent isolates, were included as far as possible.

TABLE I. Strains used in the Survey.

Survey No.	Generic and Specific names on receipt.	Designation on receipt.	Source.	Remarks.
G1	Listeria denitrificans	Seeliger L84/68		
G2	" grayi	" L56/66		
G3	" "	" W-L96/65		
G4	" sp.	Mair 24733	human	
G5	" monocytogenes	Murray 148	wolf-coyote hybrid	
G6	" "	" 149	"	
G7	" "	" 44	ferret	
G8	" sp.	" 50	cow brain	
G9	" sp.	" 51	human tonsil	
G10	" monocytogenes	" 104	artic fox	
G11	" "	" 127	wild snowshoe hare	
G12	" "	" 128	moose brain	
G13	" "	" 133	Dermacentor pictus	
G14	" "	" 134	spring water	
G15	" "	" 139	Salmo iridens	
G15a	" "	" 139	"	+
G16	" "	" 140	"	
G16a	" "	" 140	"	+
G17	" "	" 141	mud	

TABLE I. (continued.)

Survey No.	Generic and Specific name on receipt.	Designation on receipt.		Source.	Remarks.
G18	Listeria monocytogenes	Mair		human	
G19	" "	"	28251	human	
G20	" "	"	17	"	
G21	" "	"	26431	"	
G22	" "	NCTC	5348		
G23	" "	"	10357		TYPE
G24	" "	"	5105		
G25	" "	"	4885		
G26	" "	Seeliger	L88/7		Rough strain from Potel
G27	" "	Mair	17		
G28	" "	NCTC	4885		
G29	" "	"	5105		
G30	" "	"	5214		
G31	" "	"	5348		
G32	" "	"	7973		HOLOTYPE
G33	" "	"	4883		
G34	" "	Wood		calf	
G35	" "	Brzin		human	
G36	" "	Mair		"	
G37	" "	Jones		calf brain	
G38	" "	Kahn	560		

TABLE I. (continued.)

Survey No.	Generic and Specific names on receipt.	Designation on receipt.		Source.	Remarks.	
G39	Listeria monocytogenes	Kahn	611			
G40	" "	"	611		non-haemolytic	
G42	" grayi	Seeliger	L332/64	chinchilla	Larsen & Seeliger (1966)	
G43	" denitrificans	P.I.C.	55134	heated blood	L26	
G44	" murrayi	ATCC	25401		Welshimer et al.(1971)	
G45	" "	"	25402		"	
G46	" "	"	25403		"	
G47	" grayi	"	25400		"	
G48	Streptococcus sp. Grp.L.	NCTC	10238			
G60	Lactobacillus acidophilus	ATCC	4356		TYPE	*
G61	" " biotype I	"	19992			*
G62	" brevis	"	14869		TYPE	*
G63	" buchneri	"	4005		TYPE	*
G64	" bulgaricus	"	11842		TYPE	*
G65	" casei	"	393		TYPE	*
G66	" " var. rhamnosus	"	7469			*
G67	" cellobiosus	"	11739		TYPE	*
G68	" delbrueckii	"	9649			*
G69	" fermentum	"	14931			*
G70	" "	"	14932			*

TABLE I. (continued)

Survey No.	Generic and Specific names on receipt.	Designation on receipt.		Source.	Remarks.	
G71	Lactobacillus fermentum II	ATCC	23272			*
G72	" helveticus	"	15009			*
G73	" jugurt	"	521			*
G74	" "	"	7993			*
G75	" lactis	"	12315		TYPE	*
G76	" leichmannii	"	4797			*
G77	" plantarum	"	14917			*
G78	" salivarius sub sp. salivarius		11741		TYPE	*
G79	" " sub sp. salicinius		11742		COTYPE	*
G80	" sp.	NCDO	204			*
G81	" delbrueckii	NCDO	1744			*
G82	Gemella haemolysans	NCTC	10459			
G83	" "	"	10243			
G84	" "	"	10244			
G85	Neisseria "	Kellog				
G86	Microbacterium thermosphactum	Lord	1	pre-packed pork		
G87	" "	"	2	"		
G88	" "	"	3	"		
G89	" "	"	11	"		

TABLE I. (continued.)

Survey No.	Generic and Specific name on receipt.	Designation on receipt.	Source.	Remarks.
G90	Microbacterium thermosphactum	Kitchell 1A(BaA1)	beef	
G91	" "	" 124(BaA6)	"	
G92	" "	" 13(BaV3)	"	
G93	" "	" 13A "	"	
G94	" "	" 13B "	"	
G95	" "	" 144(BfA9)	"	
G96	" "	" 156(BfV9)	"	
G97	" "	" 101(LfA3)	lamb	
G98	" "	" 46(LfV1)	"	
G99	" "	" 128(LfV6)	"	
G100	" "	" WR7		ex Niven
G101	" "	" CR12a		"
G102	" "	" 30		"
G103	" "	" 30A		"
G104	" "	NCIB 10018		TYPE
G105	" "	Lord 33	pre-packed pork	
G106	" "	Gardner 44		
G107	" "	" 47		
G108	" "	" 52		
G109	" "	" 54		

TABLE I. (continued.)

Survey No.	Generic and Specific names on receipt.				Designation on receipt.		Source.	Remarks.
G110	Microbacterium thermosphactum				Gardner	58		
G111	Kurthia	zopfii			"	26	hamburger	
G112	"	"			"	33	frozen minced pork	
G113	"	"			"	38	pork sausage	
G114	"	"			"	31	fat trimmings	
G115	"	"			"	34	rendered lard	
G116	"	"			"	76	pork sausage	
G117	"	"			Keddie	K2	milk	
G118	"	"			"	S8	meat	
G119	"	"			"	S9	meat	
G120	Streptococcus pyogenes		Grp	A	NCTC	8198		TYPE
G121	"	agalactiae	"	B	"	8181		TYPE
G122	"	equi	"	C	"	9682		
G123	"	bovis	"	D	"	8177		
G124	"	durans	"	D	"	8307		TYPE
G125	"	faecalis	"	D	"	775		
G126	"	faecium	"	D	"	7171		TYPE
G127	"	liquifaciens	"	D	"	8175		
G128	"	zymogenes	"	D	"	8176		
G129	"	sp.	"	E	"	5385		

TABLE I. (continued.)

Survey No.	Generic and Specific name on receipt.		Designation on receipt.		Source.	Remarks.
G130	Streptococcus sp.	Grp. F	NCTC	5389		
G131	" sp.	Grp. G	"	8547		
G132	" sp.	Grp. H	"	7868		
G1330	" sp.	Grp. K	"	5338		Opaque"variant"
G133T	" sp.	Grp. K	"	"		Translucent"variant"
G134	" sp.	Grp. Q	"	9938		
G135	" sp.	Grp. M	"	7760		
G136	" sp.	Grp. N	"	6681		
G137	" sp.	Grp. O	"	8029		
G138	" sp.		Board		sausage	
G139	" uberis		NCTC	3858		TYPE
G140	" equinis		ATCC	9812		
G141	" suis		Elliott	PM1		
G142	Lactobacillus mali		Carr	12	cider	Carr & Davies (1970)
G143	" "		"	123	"	"
G144	" "		"	26	"	"
G145	Cellulomonas biazotea		NCIB	8077		TYPE
G146	" fimi		"	8980		
G147	" flavigena		"	8073		COTYPE
G148	" bibula		"	8142		
G149	" rossica		"	8074		

TABLE I. (continued.)

Survey No.	Generic and Specific names on receipt.	Designation on receipt.	Source.	Remarks.
G150	Cellulomonas uda	ATCC	491	TYPE
G151	Propionibacterium shermanii	NCIB	8099	TYPE
G152	" freundenreichii	"	5959	
G153	" peterssonii	"	5962	TYPE
G154	" pentosaceum	"	8070	TYPE
G155	Corynebacterium fascians	NCPPB	1488	
G156	" insidiosum	"	83	
G157	" rathayi	"	797	
G158	" poinsettiae	"	844	
G159	" diphtheriae	NCTC	3984	TYPE
G160	" manihot	NCIB	9097	
G161	" mediolanum	"	7206	
G162	" michiganense	NCPPB	1468	
G163	" betae	"	363	
G164	Brevibacterium acetylicum	ATCC	953	TYPE
G165	" linens	"	9174	COTYPE
G166	" stationis	"	14403	
G167	" leucinophagum	"	13809	TYPE
G168	Kurthia zopfii	NCTC	404	
G169	Brevibacterium imperiale	ATCC	8365	TYPE

TABLE I. (continued.)

Survey No.	Generic and Specific name on receipt.	Designation on receipt.	Source.	Remarks.
G170	Brevibacterium ammoniagenes	NCIB 8143		TYPE
G171	" divaricatum	" 9397		
G172	Bacterium eurydice	Bailey	honey bees	
G173	" "	"	"	
G174	" "	"	"	
G175	Staphylococcus epidermidis	NCTC 7291		
G176	" saprophyticus	" 7292		TYPE
G177	" aureus	" 8532		TYPE
G178	" "	" 6571		Also known as "Oxford"
G179	Micrococcus luteus	" 8512		TYPE
G180	" hyicus	" 10350		TYPE
G181	" agilis	" 2676		TYPE
G182	" denitrificans	NCIB 8944		TYPE
G183	Erysipelothrix rhusiopathiae	Wellcome CN571		from Woodbine
G184	" "	" CN354		"
G185	" "	" CN446		"
G186	" "	" CN906		"
G187	" "	" CN2432		"

TABLE I. (continued.)

Survey No.	Generic and Specific names on receipt.	Designation on receipt.	Source.	Remarks.
G188	Erysipelothrix rhusiopathiae	Wellcome CN6260		from Woodbine
G189	" "	London Zoo S52	pheasant	
G190	" "	NCTC 8163		TYPE
G191	" "	Sneath 155	human	
G192	" "	" 13	"	
G193	" "	" 49	"	
G194	" "	" 19	"	
G195	" "	" 45	"	
G196	Streptococcus suis	Elliott PM35	pig	
G197	" "	" PM37	pig	
G198	" "	" 596/70		originally from Bertschinger
G199	" "	" 627/70		"
G200	" "	" 34/70		"
G201	Arthrobacter sp.	Hill A137/68	throat	
G202	" sp.	Brzin		

+ On receipt the cultures contained haemolytic and non-haemolytic colonies, these were separated and treated as different strains.

TABLE I. (continued)

* These strains were suggested as neotypes by The Taxonomic Subcommittee on Lactobacilli and Closely Related Organisms, a subcommittee of The International Committee on Nomenclature of Bacteria, of The International Association of Microbiological Societies.

TYPE This designation is given to those strains which are listed as Type Strains by Sneath, P.H.A. and Skerman, V.B.D. (1966).

COTYPE This designation is given to those strains which are listed as Cotypes by Sneath, P.H.A. and Skerman, V.B.D. (1966).

ADDRESSES OF SUPPLIERS OF STRAINS.

ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, U.S.A.

Bailey, L., Rothamsted Experimental Station, Harpenden, Herts.

Board, R.G., University of Bath, Claverton Down, Bath, Somerset.

Brzin, B., St. Vincent's Hospital, Adelaide, Australia.

TABLE I. (continued.)

ADDRESSES OF SUPPLIERS OF STRAINS. (continued.)

Carr, J.G., Cider and Fruit Juices Division, Research Station, Longashton, Bristol.

Elliott, S.D., Department of Pathology, University of Cambridge, Cambridge, CB2 1PQ.

Gardner, G.A., Ulster Curers Association, 2, Greenwood Avenue, Belfast, BT4 3JL.

Hill,L.R., Central Public Health Laboratory, Colindale Avenue, London.

Jones, D., M.R.C., Microbial Systematics Unit, Leicester University, Leicester, LE1 7RH.

Kahn, M.A., University of Nottingham, Department of Microbiology, Sutton Bonnington.

Kellogg, D.S., Communicable Disease Centre, Venereal Disease Research Laboratory, Atlanta, Ga. 30333, U.S.A.

Keddie, R.M., Department of Microbiology, University of Reading, London Road, Reading.

Kitchell, A.G., Meat Research Institute, Langford, Bristol, BS18 7DY.

London Zoo, Regents Park, London.

Lord, A., M.R.C., Microbial Systematics Unit, Leicester University, Leicester LE1 7RH.

Mair, N.S., Public Health Laboratory, Leicester.

TABLE I. (continued.)

ADDRESSES OF SUPPLIERS OF STRAINS. (continued.)

Murray, E.G.D.(The Late), via R.G.E. Murray, Department of Bacteriology and Immunology, The University of Western Ontario, London 72, Canada.

NCDO, National Collection of Dairy Organisms, National Institute for Research in Dairying, Shinfield,Reading, Berks.

NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen.

NCPPB, National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Ministry of Agriculture, Hatching Green, Harpenden, Herts.

NCTC, National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London.

PIC, Pasteur Institute Collection, Institut Pasteur, 28, Rue du Docteur Roux, Paris 15, France.

Seeliger, H.P.R., Instute for Hygiene and Microbiology, University of Wurzburg, Germany.

Sneath, P.H.A., M.R.C., Microbial Systematics Unit, Leicester University, Leicester.

TABLE I. (continued.)

ADDRESSES OF SUPPLIERS OF STRAINS. (continued.)

Wellcome, The Wellcome Foundation, Beckenham, Kent, BR3 3BS.

Wood, D., M.R.C., Microbial Systematics Unit, Leicester University, Leicester, LE1 7RH.

Woodbine, M., University of Nottingham, Department of Microbiology, Sutton Bonnington.

APPENDIX II.

MEDIA AND TEST REAGENTS.

MEDIA AND TEST REAGENTS.

I Media.

All media were autoclaved at 121°C for 15 mins. unless otherwise stated. Distilled water was used in all instances.

a) Basal Media

Basal Medium agar. (BM agar)

This was modified from MRS agar, Oxoid CM361, by omission of citrate and acetate.

Peptone	Oxoid L34	10g.
Lab Lemco	" L29	8g.
Yeast Extract	" L21	4g.
Glucose		20g.
Tween 80		1ml.
Dipotassium hydrogen orthophosphate		2g.
Magnesium sulphate ($MgSO_4.7H_2O$)		0.2g.
Manganese sulphate ($MnSO_4.5H_2O$)		0.05g.
Agar	Oxoid No.1	10g.
Distilled water		1000ml.

Basal Medium broth. (BM broth)

As BM agar, but with the omission of the agar.

Sugar Basal Medium agar. (SBM agar)

Peptone	Oxoid L34	15g.
Yeast Extract	" L21	1g.
Tween 80		1ml.
Dipotassium hydrogen orthophosphate		2g.
Magnesium sulphate ($MgSO_4.7H_2O$)		0.2g.

Manganese sulphate ($MnSO_4.7H_2O$)		0.05g.
Agar	Oxoid No.1	10g.
Distilled water		1000ml.

Sugar Basal Medium broth (SBM broth)

As SBM agar, but with the omission of the agar.

b) Other Media.

Aesculin broth. ref: Sneath (1956).

SBM broth	1000ml.
Sodium citrate	1g.
Aesculin	1g.
Ferric citrate scales	0.05g.

The ferric citrate scales were dissolved by boiling in a small volume of water, and added to the rest of the medium, which was then dispensed into bijoux bottles in 3-4ml. amounts before sterilization.

Arginine medium. ref: Thornley (1960).

SBM broth	1000ml.
Arginine hydrochloride	10g.
Phenol red (2%w/v aq. solution)	5ml.
Agar Oxoid No.1	3g.

The sterilized medium was dispensed aseptically in 4ml. amounts into bijoux bottles. Liquid paraffin was sterilized at 160°C for 1 hour, and layered on the agar in those bottles to be used for anaerobic studies.

Casein agar.

BM agar with twice the normal concentration of agar, and no glucose was sterilized at 121°C for 15 mins. A 10%w/v solution of Marvel skimmed milk was sterilized at 121°C for 5mins.

Equal volumes of each were mixed after cooling to 60°C, and poured into petri dishes.

DNase agar. ref: Di Salvo (1958)

BM agar	1000ml.
Deoxyribonucleic acid	2g.
Calcium chloride (anhydrous)	0.8g.

The calcium chloride was thoroughly dissolved in the basal medium before the DNA was added, to prevent any precipitation, and after sterilization the medium was poured into petri dishes.

Glucose broth. ref: Davis (1955)

SBM broth	1000ml.
Brom-cresol purple (2%w/v aq. solution)	1.5ml.
Glucose	1g.

The broth was dispensed into 6"x$\frac{5}{8}$" tubes containing inverted Durham tubes, and sterilized at 114°C for 10 mins.

Glucose-Lemco broth. ref: Jones and Shattock (1960)

Peptone (Evans)		10g.
Lab Lemco	Oxoid L29	10g.
Sodium chloride		5g.
Glucose		5g.
Distilled water		1000ml.

The broth was dispensed into 100 ml. bottles, and sterilized as usual.

Hippurate broth. ref: Davis (1955)

SBM broth	1000ml.
Glucose	1g.
Sodium hippurate	10g.

10 ml. quantities were dispensed into universal bottles before sterilization.

Litmus milk. ref: Wheater (1955)

Litmus milk	Oxoid CM 45	100g.
Yeastrel		3g.
Glucose		10g.
Distilled water		1000ml.

The broth was filtered through muslin and then distributed in 10 ml. amounts into universal bottles. Sterilization was acheived by heating at 121°C for 5 mins.

MRVP medium. ref: Barritt (1936)

BM broth plus reduced glucose (5 g./l.) and increased di-potassium hydrogen orthophosphate (5 g./l.), was dispensed into universal bottles in 12.5 ml. amounts, and sterilized at 114°C for 20 mins.

Nitrate broth. ref: Smith and Cunningham (1962)

BM broth	1000ml.
Potassium nitrate	1g.

10 ml. quantities were dispensed into 6"x$\frac{5}{8}$" tubes containing inverted Durham tubes.

Nitrite broth.

BM broth	1000ml.
Potassium nitrite	1g.

5ml. quantities were dispensed into 6"x$\frac{5}{8}$" tubes and then sterilized.

"O" and "F" test medium. ref: Hugh and Leifson (1953)

BM broth	1000ml.
Brom-cresol purple (2%w/v aq. solution)	1.5ml.

Agar	Oxoid No. 1	4g.

The medium was sterilized, cooled to 60°C and to it was added 40 ml. of a 50%w/v aq., filter sterilized solution of glucose. 10 ml. quantities of the medium were aseptically dispensed into 6"x$\frac{5}{8}$" tubes, and when set, 2 ml. of sterile paraffin was added to those tubes to be used for anaerobic studies.

Ouchterlony agar. ref: Ouchterlony (1953)
Mansi (1958)

Purified agar	Oxoid L28	10g.
Sodium chloride		8.5g.
Sodium azide		1g.
Distilled water		1000ml.

Phosphatase agar. ref: Edwards and Jones (1966)

BM agar	1000ml.
Phenolphthalein diphosphate (tetra sodium salt) B.D.H. (1%w/v aq. solution)	10ml.

The BM agar was sterilized and cooled to 60°C, the filter sterilized phenolphthalein diphosphate solution was then added, and the agar poured into petri dishes.

Potassium tellurite agar.

BM agar was sterilized and cooled to 60°C. A 1%w/v aq. solution of potassium tellurite was sterilized by autoclaving at 121°C for 5 mins., and then added to the cooled agar to final concentrations of 0.01%, 0.025% and 0.05%w/v. The agar was then dispensed into divided dishes.

Potassium thiocyanate agar.

BM agar plus 2.5%, 3.75% and 4.5%w/v potassium thiocyanate was sterilized and dispensed into divided dishes.

Sharpe's Medium A. ref: Sharpe(1955)

Yeastrel		3g.
Glucose		20g.
Sodium chloride		5g.
Tween 80		0.1g.
Peptone	Evans	15g.
Distilled water		1000ml.

The broth was dispensed into 500 ml. screw-top bottles before sterilization.

Sodium azide agar.

BM agar plus 0.01%, 0.02% and 0.05%w/v sodium azide was sterilized and dispensed into divided dishes.

Starch agar ref: Allen (1918)

BM agar lacking glucose	1000ml.
Starch	2g.

The starch was dissolved by heating in a small quantity of the distilled water, and then added to the BM agar. After sterilization the agar was poured into petri dishes.

Tetrazolium agar.

SBM agar was sterilized and cooled to below 60°C, a filter sterilized 10%w/v aq. solution of 2,3,5-triphenyl tetrazolium chloride was added to final concentrations of 0.1% and 0.01% w/v, and a filter sterilized solution of glucose to a final concentration of 1%w/v. The agar was then dispensed into divided dishes.

Thallous acetate agar.

BM agar plus 0.01%, 0.02% and 0.03%w/v thallous acetate was sterilized, and dispensed into divided dishes.

Tryptone broth. ref: Smith and Cunningham (1962)

Tryptone Oxoid L42	20g.
Dipotassium hydrogen orthophosphate	2g.
Glucose	2g.
Magnesium sulphate ($MgSO_4.7H_2O$)	0.2g.
Manganese sulphate ($MnSO_4.5H_2O$)	0.05g.
Distilled water	1000ml.
Final pH	7.2.

7 ml. amounts of the broth were dispensed into 6"x$\frac{5}{8}$" tubes and then sterilized.

Tween agar.

BM agar was modified by reducing the amount of glucose to 10g/l. and by replacing the Tween 80 normally used, by 10 ml. per l. of the Tween to be tested. Tweens 20, 40, 60 and 80 were used, and after sterilization the agar was poured into petri dishes.

Tyrosine agar.

BM agar minus glucose	1000ml.
L-tyrosine	5g.

After sterilization, the agar was poured into petri dishes.

Urea agar. ref: Christensen (1946)

BM agar plus reduced glucose (0.5%w/v)	1000ml.
Phenol red (2%w/v aq. solution)	5ml.
Final pH	6.8.
Urea (50%w/v aq. solution)	40ml.

The BM agar plus phenol red was sterilized and cooled to 60°C, the urea solution was filter sterilized and added to the agar. The medium was then dispensed aseptically in 5ml. amounts

into 6"x$\frac{5}{8}$" tubes which were then slanted to produce slopes.

Xanthine agar.

BM agar minus glucose	1000ml.
Xanthine	5g.

After sterilization the agar was poured into petri dishes.

II Test Reagents.

Kovács' Reagent. ref: Kovács (1928)

p-dimethylaminobenzaldehyde	5g.
Amyl alcohol	75ml.
Concentrated hydrochloric acid	25ml.

The aldehyde was dissolved in the alcohol by heating in a water-bath at 55°C. When cool, the acid was added, and the reagent stored at 4°C.

Nitrate Reagent A. (Griess Ilosvay reagent)

α-naphthylamine	1g.
Distilled water	22ml.
25% acetic acid	180ml.

The α-naphthylamine was dissolved in the water, the solution filtered, and then the acetic acid was added. Storage was at 4°C.

Nitrate Reagent B. (Griess-Ilosvay reagent)

Suphanilic acid	0.5g.
25% acetic acid	150ml.

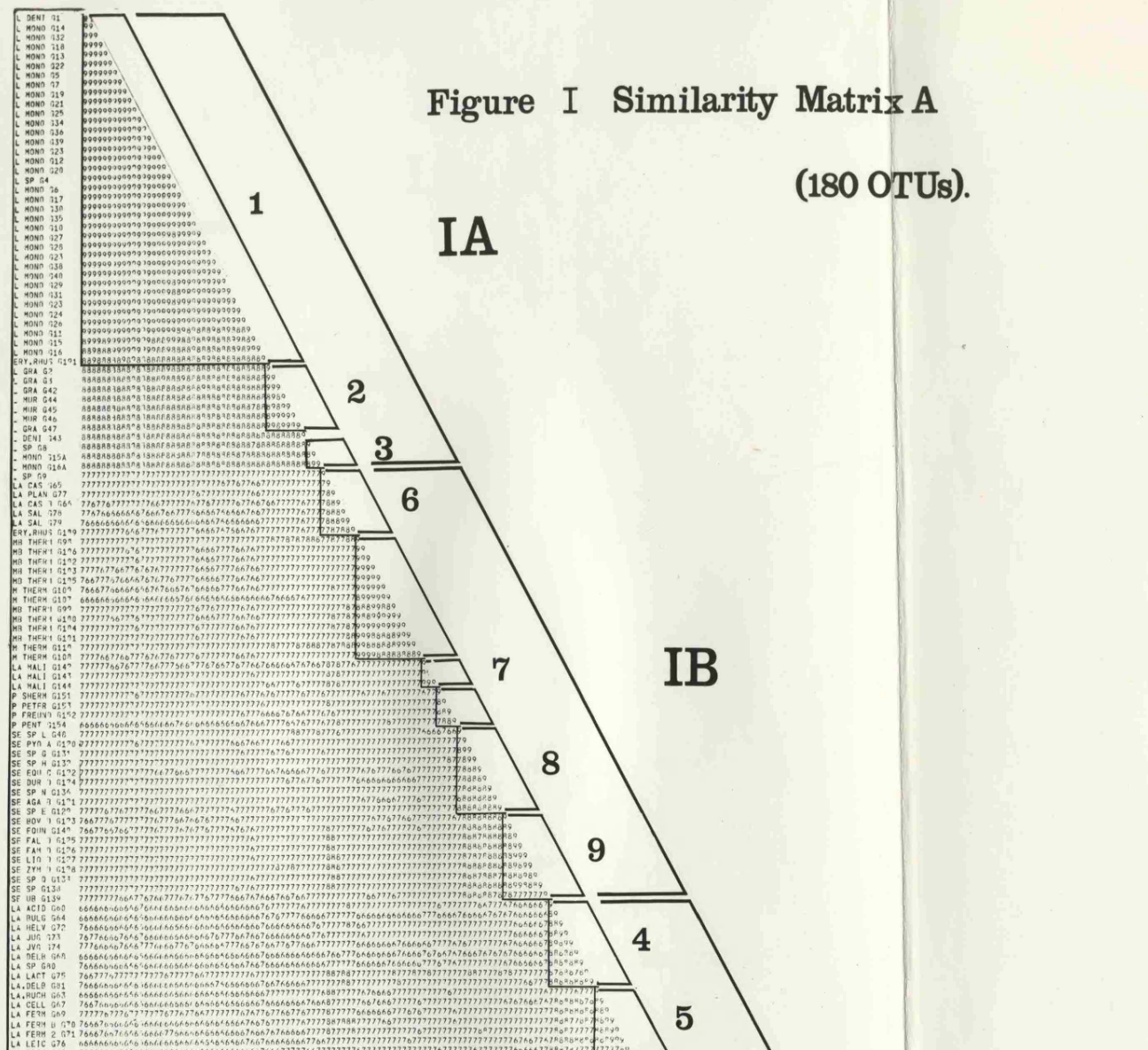
The sulphanilic acid was dissolved in the acetic acid, and the solution stored at 4°C.

APPENDIX III.

THE SIMILARITY MATRICES AND TABLE OF CLUSTER CHARACTERISTICS PRODUCED IN THE NUMERICAL TAXONOMIC SURVEY.

Figure I Similarity Matrix A

(180 OTUs).



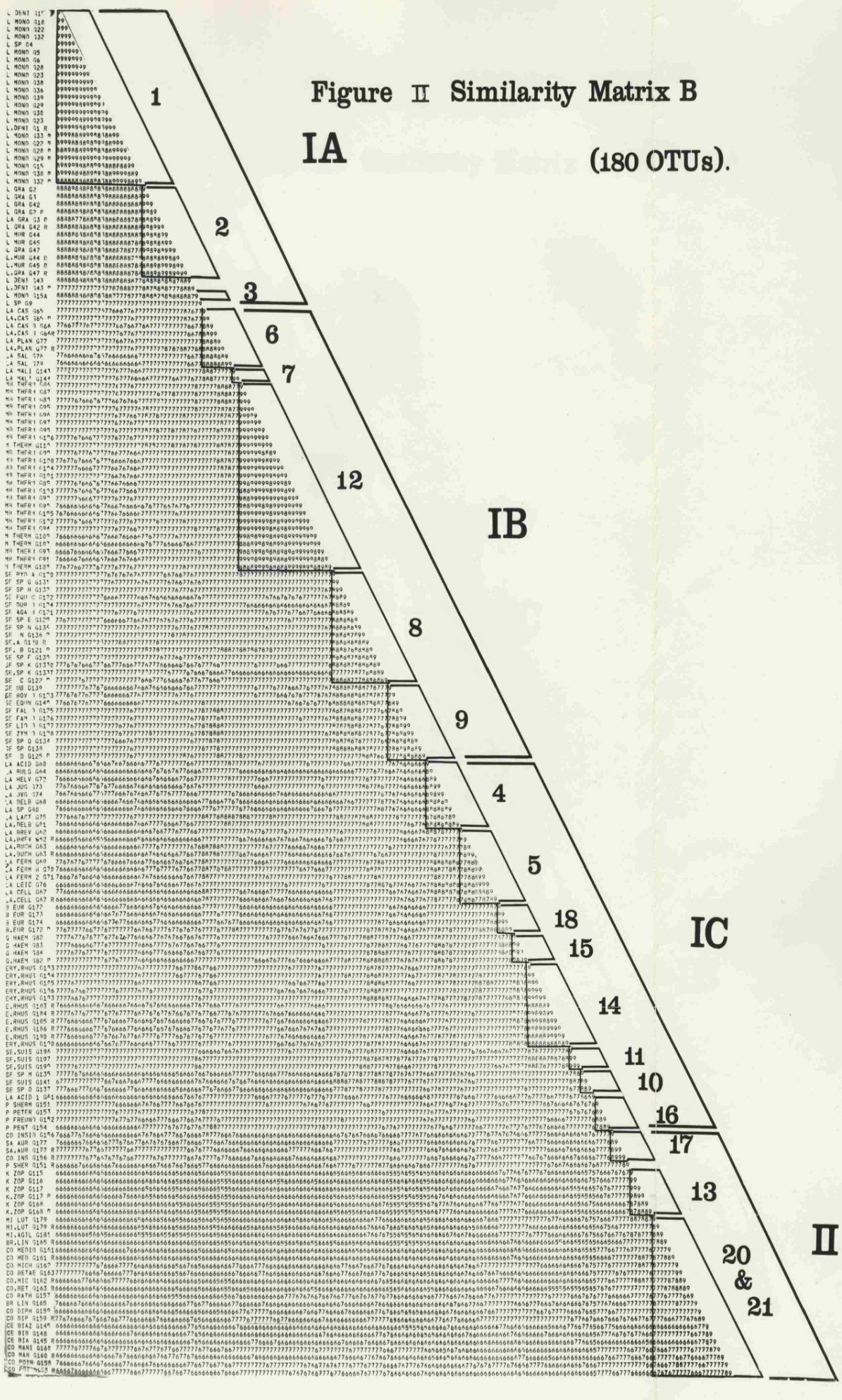
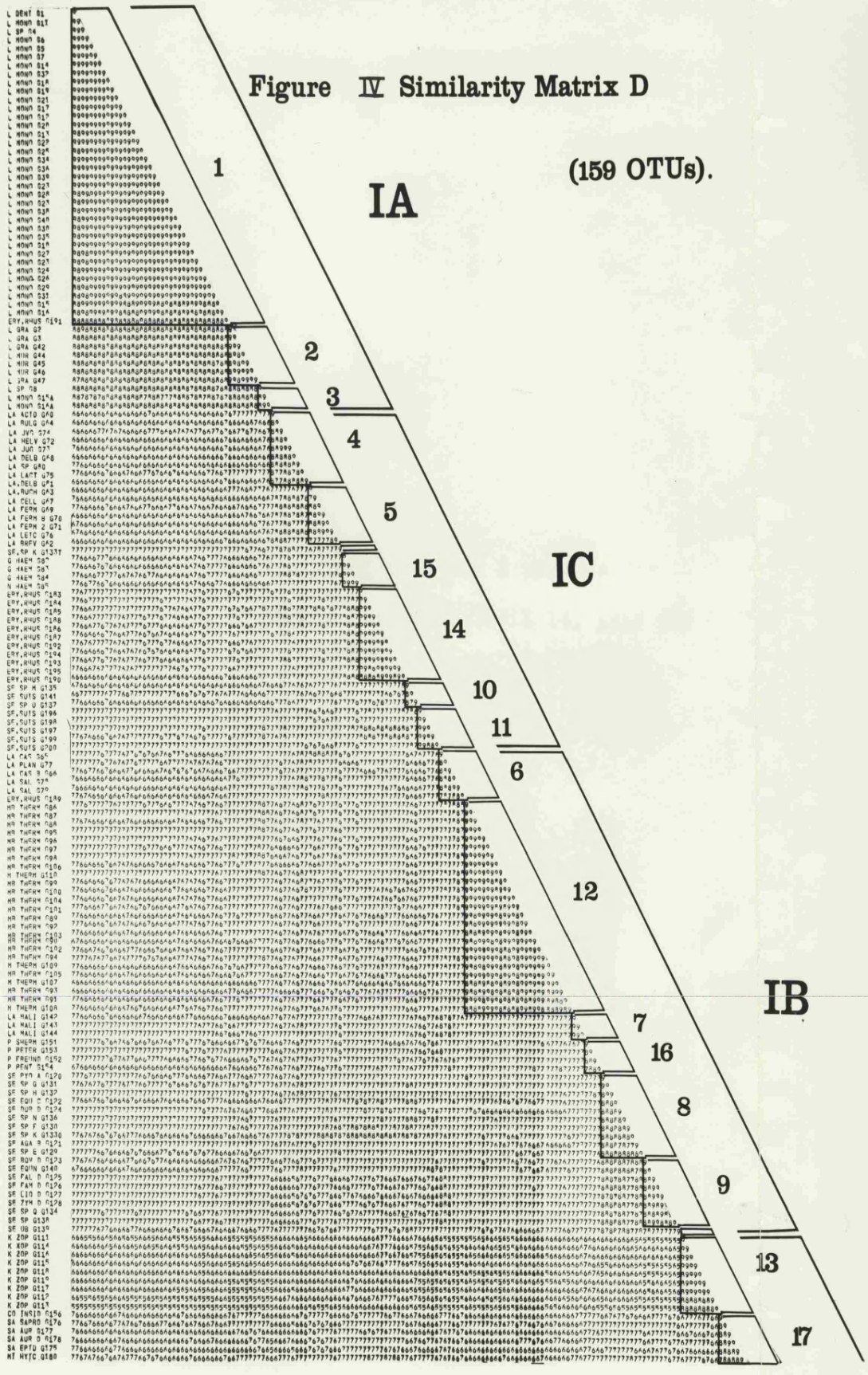


Figure II Similarity Matrix B
IA
(180 OTUs).
IB
IC
II
1
2
3
6
7
12
8
9
4
5
18
15
14
11
10
16
17
13
20
&
21

Figure IV Similarity Matrix D

(159 OTUs).



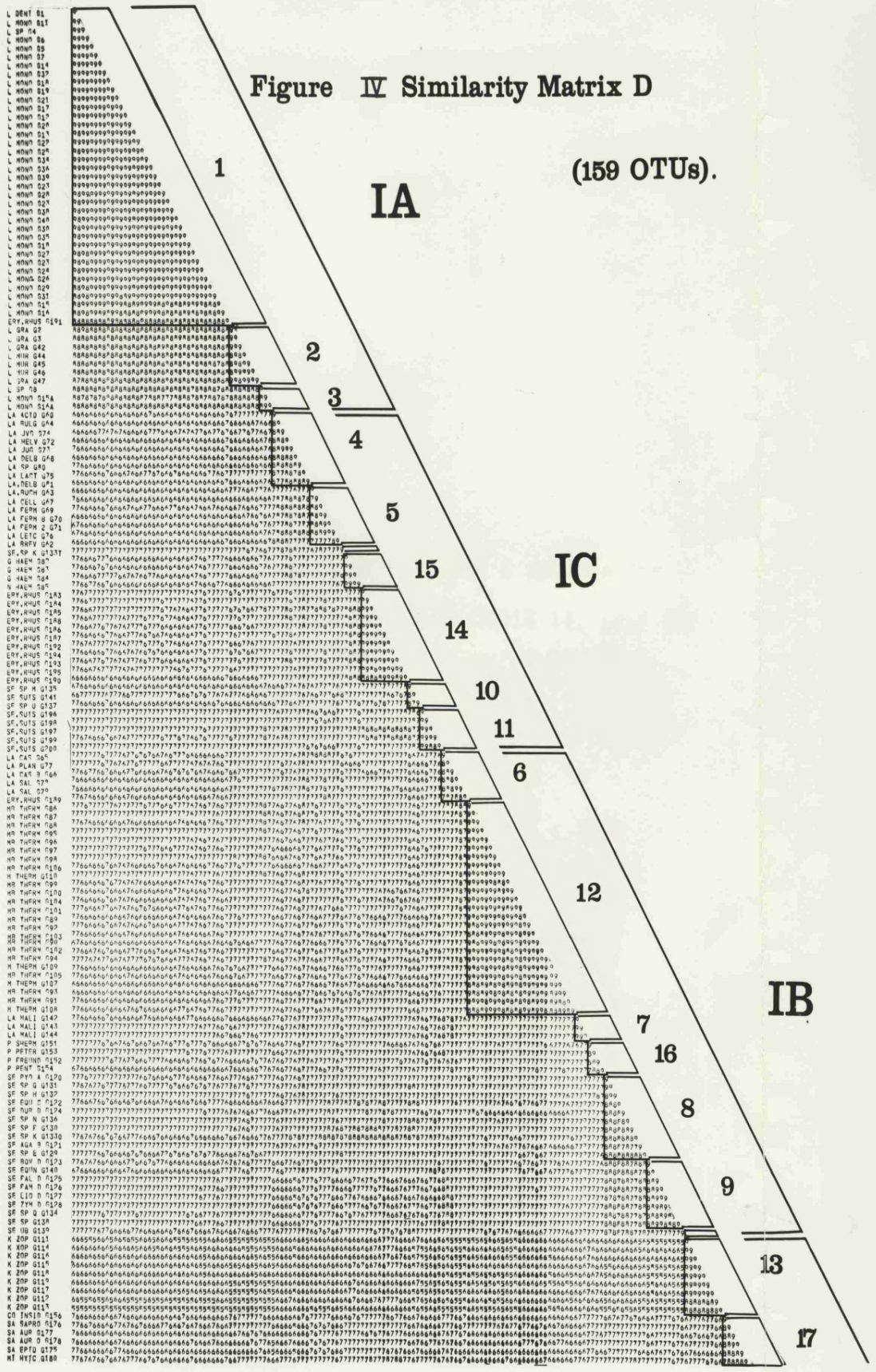


Figure IV Similarity Matrix D
(159 OTUs).
IA
IB
IC
II
1
2
3
4
5
15
14
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12
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16
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17

TABLE I. Distribution of 0, 1 and 2 results for each of the 15 groups listed in TABLE 14, page 96.

Characters	1 Listeria monocytogenes 1			2 Listeria murrayi / grayi			3 Listeria monocytogenes 2			4 Lactobacillus 1			5 Lactobacillus 2			6 Lactobacillus 3			7 Lactobacillus mali			8 Streptococcus 1		
	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
1 Smooth / Rough	36	1	0	7	0	0	2	1	0	8	1	0	6	1	0	5	1	0	0	3	0	11	0	0
2 Entire / Serrated	8	29	0	1	6	0	0	3	0	0	9	0	2	5	0	1	5	0	0	3	0	10	1	0
3 Elevation - low convex	33	4	0	5	2	0	3	0	0	9	0	0	5	2	0	4	2	0	0	3	0	1	10	0
4 - convex	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
5 - fried egg	2	35	0	1	6	0	0	3	0	0	9	0	2	5	0	2	4	0	3	0	0	10	1	0
6 Consistency - watery	2	35	0	0	7	0	0	3	0	8	1	0	2	5	0	1	5	0	1	2	0	2	9	0
7 - butyrous	36	1	0	7	0	0	3	0	0	1	8	0	5	2	0	5	1	0	2	1	0	9	2	0
8 - viscous	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
9 - bitty	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
10 Translucent colony	0	37	0	4	3	0	0	3	0	0	9	0	0	7	0	1	5	0	3	0	0	2	9	0
11 Homogeneous emulsification	0	37	0	0	7	0	0	3	0	1	8	0	1	6	0	0	6	0	1	2	0	8	3	0
12 Pigmentation - yellow	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
13 - pink	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
14 Gram positive	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	1	10	0
15 Cell form - long rods	37	0	0	7	0	0	3	0	0	0	9	0	0	7	0	3	3	0	0	3	0	11	0	0
16 - short rods	36	1	0	2	5	0	2	1	0	6	3	0	3	4	0	1	5	0	0	3	0	11	0	0
17 - coccobacilli	0	37	0	0	7	0	0	3	0	9	0	0	6	1	0	5	1	0	3	0	0	11	0	0
18 - cocci	37	0	0	5	2	0	3	0	0	9	0	0	7	0	0	5	1	0	3	0	0	0	11	0
19 - filaments	35	2	0	7	0	0	1	2	0	3	6	0	3	4	0	4	2	0	0	3	0	11	0	0
20 - chains	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	1	5	0	0	3	0	0	11	0
21 Adherence	8	29	0	1	6	0	1	2	0	8	1	0	7	0	0	6	0	0	3	0	0	6	5	0
22 Haemolysis - alpha	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	10	1	0
23 - beta	3	34	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	2	9	0
24 Colony diameter <1/1-3/>3mm	8	29	0	0	7	0	0	3	0	9	0	0	7	0	0	5	1	0	3	0	0	6	5	0
25 Pleomorphism	37	0	0	7	0	0	3	0	0	6	3	0	4	3	0	6	0	0	3	0	0	11	0	0
26 Rhizoidal margin	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	3	8	0
27 Pellet - stringy	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	1	5	0	0	3	0	1	10	0
28 - granular	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	5	1	0	3	0	0	11	0	0
29 - floculent	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	10	1	0
30 Pellicle? Ring/ neither	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
31 Easy pellet resuspension	0	37	0	0	7	0	0	3	0	0	9	0	1	6	0	1	5	0	0	3	0	4	7	0
32 Beaded Gram stain	37	0	0	7	0	0	3	0	0	0	9	0	0	7	0	0	6	0	0	3	0	11	0	0
33 Motile	2	35	0	4	3	0	3	0	0	8	1	0	7	0	0	6	0	0	0	3	0	0	11	0
34 Tumbling	2	35	0	4	3	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
35 Cellulose hydrolysis	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
36 Tween 20 "	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
37 Tween 40 "	37	0	0	7	0	0	3	0	0	8	1	0	6	1	0	5	1	0	3	0	0	10	1	0
38 Tween 60 "	4	33	0	7	0	0	3	0	0	6	3	0	3	4	0	0	6	0	0	3	0	1	10	0
39 Tween 80 "	37	0	0	7	0	0	3	0	0	8	1	0	7	0	0	6	0	0	3	0	0	11	0	0
40 Tyrosine "	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	10	1	0
41 Xanthine "	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
42 Growth at 5°C	0	1	36	0	0	7	0	0	3	5	3	1	0	6	1	1	2	3	0	0	3	6	3	2
43 " 10°C	1	0	36	0	0	7	0	0	3	2	6	1	0	4	3	1	1	4	0	0	3	0	9	2
44 " 20°C	0	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11
45 " 30°C	0	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11
46 " 35°C	0	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11
47 " 45°C	0	17	20	0	0	7	0	3	0	1	1	7	0	3	4	0	0	6	1	2	0	0	9	2
48 " 50°C	36	0	1	7	0	0	3	0	0	6	1	2	4	3	0	6	0	0	3	0	0	10	0	1
49 Gluconate oxidation	37	0	0	7	0	0	3	0	0	9	0	0	6	1	0	5	1	0	3	0	0	11	0	0
50 Indole from tryptone	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
51 Lecithinase activity	6	31	0	1	6	0	2	1	0	1	8	0	3	4	0	0	6	0	0	3	0	3	8	0
52 Methyl Red Test	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0
53 Voges-Proskauer Test	0	36	1	0	7	0	0	3	0	8	0	1	7	0	0	0	5	1	3	0	0	10	1	0
54 Inhib'n by Thallous acetate 0.01%	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
55 " 0.02%	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
56 " 0.03%	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
57 Catalase activity	1	36	0	0	7	0	3	0	0	9	0	0	7	0	0	6	0	0	0	3	0	10	1	0
58 Oxidase activity	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
59 Growth under hydrogen	1	36	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0
60 Inhib'n by sodium chloride 6.5%	35	2	0	7	0	0	1	2	0	0	1	8	4	1	2	4	2	0	0	2	1	3	7	1
61 " 10 %	12	24	1	7	0	0	0	3	0	0	1	8	0	3	4	0	2	4	0	2	1	0	6	5
62 Red'n of potassium tellurite 0.01 %	0	37	0	0	7	0	0	3	0	6	3	0	1	6	0	1	5	0	0	3	0	0	11	0
63 " " 0.025%	0	37	0	0	7	0	0	3	0	8	1	0	1	6	0	4	2	0	1	2	0	1	10	0
64 " " 0.05 %	0	37	0	0	7	0	0	3	0	7	2	0	1	6	0	4	2	0	2	1	0	3	8	0
65 Inhib'n by tetrazollium 0.01%	37	0	0	7	0	0	3	0	0	8	1	0	2	5	0	5	1	0	3	0	0	11	0	0
66 Red'n of " 0.01%	0	37	0	0	7	0	0	3	0	2	7	0	0	7	0	1	5	0	3	0	0	0	11	0
67 Inhib'n by " 0.1 %	36	0	1	7	0	0	3	0	0	0	5	4	0	6	1	0	4	2	0	3	0	2	8	1
68 Red'n of " 0.1 %	0	37	0	0	7	0	0	3	0	6	3	0	1	6	0	4	2	0	3	0	0	2	9	0
69 Inhib'n by pot. thiocyanate 2.5%	37	0	0	7	0	0	3	0	0	0	4	5	0	7	0	0	6	0	0	3	0	0	4	7
70 " " 3.7%	37	0	0	7	0	0	3	0	0	0	2	7	0	7	0	0	6	0	0	3	0	0	1	10
71 " " 4.5%	37	0	0	7	0	0	3	0	0	0	2	7	0	6	1	0	3	3	0	3	0	0	0	11
72 " sodium azide 0.01%	0	6	31	1	6	0	0	2	1	7	2	0	7	0	0	6	0	0	0	3	0	11	0	0
73 " " 0.02%	0	3	34	0	7	0	0	1	2	4	5	0	7	0	0	6	0	0	0	0	3	4	7	0
74 " " 0.05%	0	1	36	0	1	6	0	0	3	1	6	2	3	4	0	4	2	0	0	0	3	1	4	6
75 Ammonia from peptone	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
76 Hippurate hydrolysis	0	37	0	4	3	0	0	3	0	9	0	0	6	1	0	3	3	0	1	2	0	9	2	0
77 Arginine deamination (+oxygen)	36	1	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
78 " (-oxygen)	37	0	0	7	0	0	2	1	0	9	0	0	1	6	0	6	0	0	3	0	0	2	9	0
79 Aesculin hydrolysis	0	37	0	0	7	0	0	3	0	8	1	0	6	1	0	1	5	0	0	3	0	4	7	0
80 Sulphatase activity	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
81 Phosphatase activity	0	37	0	0	7	0	1	2	0	6	3	0	1	6	0	0	6	0	0	3	0	0	11	0
82 DNase "	0	37	0	0	7	0	0	3	0	0	9	0	3	4	0	3	3	0	2	1	0	0	11	0
83 RNase "	0	37	0	0	7	0	0	3	0	1	8	0	4	3	0	4	2	0	3	0	0	0	11	0
84 Gas from glucose	37	0	0	7	0	0	3	0	0	9	0	0	1	6	0	6	0	0	3	0	0	11	0	0
85 Urease activity	37	0	0	7	0	0	2	1	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
86 Hydrogen sulphide production	37	0	0	3	4	0	3	0	0	4	5	0	4	3	0	1	5	0	0	3	0	5	6	0
87 Inhib'n by Penicillin	0	6	31	0	0	7	0	2	1	0	3	6	0	4	3	0	6	0	1	2	0	0	8	3
88 " Streptomycin	0	5	32	0	6	1	0	2	1	0	3	6	0	6	1	1	5	0	0	3	0	0	9	2
89 " Chloramphenicol	0	2	35	0	0	7	0	2	1	0	4	5	0	6	1	0	6	0	0	3	0	0	8	3
90 " Chlortetracycline	0	0	37	0	0	7	0	1	2	0	4	5	0	5	2	0	6	0	0	3	0	0	8	3
91 " Oxytetracycline	0	3	34	0	0	7	0	2	1	0	6	3	0	7	0	0	6	0	0	3	0	0	10	1
92 " Erythromycin	0	1	36	0	0	7	0	2	1	0	4	5	0	6	1	0	6	0	0	3	0	0	10	1
93 " Tetracycline	0	5	32	0	0	7	0	1	2	0	6	3	0	7	0	0	6	0	0	3	0	0	9	2
94 " Sulphanilamide	36	1	0	7	0	0	3	0	0	9	0	0	6	1	0	6	0	0	3	0	0	6	5	0
95 " Novobiocin	0	14	23	0	0	7	1	2	0	0	9	0	0	7	0	0	6	0	0	3	0	0	10	1
96 " Oleandomycin	0	4	33	0	0	7	0	1	2	0	5	4	0	6	1	0	6	0	0	3	0	0	10	1
97 " Naladixic acid	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	10	1	0
98 Stimul'n by Sulphanilamide	12	25	0	4	3	0	3	0	0	8	1	0	6	1	0	6	0	0	2	1	0	11	0	0
99 Survival at 60°C for 15 min.	25	12	0	4	3	0	3	0	0	2	7	0	1	6	0	3	3	0	0	3	0	7	4	0
100 Nitrate red'n to nitrite	37	0	0	4	3	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
101 " " nitrogen	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
102 Growth in the presence of nitrite	2	35	0	1	6	0	0	3	0	9	0	0	7	0	0	6	0	0	3	0	0	10	1	0
103 Starch hydrolysis	34	3	0	2	5	0	3	0	0	0	9	0	0	7	0	0	6	0	3	0	0	6	5	0
104 Casein "	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
105 Glucose breakdown (+oxygen)	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
106 " " (-oxygen)	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0
107 Acid from inulin	36	1	0	4	3	0	0	0	3	6	2	1	7	0	0	1	5	0	1	2	0	6	4	1
108 " inositol	33	4	0	2	5	0	0	2	1	7	2	0	7	0	0	2	3	1	3	0	0	9	2	0
109 " dulcitol	35	2	0	2	5	0	1	1	1	7	2	0	7	0	0	1	5	0	1	2	0	8	3	0
110 " lactose	2	5	30	0	0	7	0	0	3	2	1	6	1	1	5	0	0	6	0	3	0	2	0	9
111 " -methyl-glucoside	0	1	36	0	7	0	0	0	3	5	3	1	5	1	1	1	4	1	0	0	3	5	3	3
112 " melibiose	33	1	3	1	0	6	2	1	0	5	2	2	2	0	5	0	2	4	1	2	0	7	4	0
113 " salicin	0	0	37	0	0	7	0	0	3	6	0	3	7	0	0	0	1	5	0	0	3	3	0	8
114 " adonitol	20	17	0	0	7	0	2	1	0	8	1	0	7	0	0	3	3	0	0	3	0	7	4	0
115 " arabinose	37	0	0	6	1	0	2	1	0	9	0	0	4	0	3	5	0	1	3	0	0	9	0	2
116 " mannitol	34	0	3	0	0	7	0	0	3	9	0	0	6	0	1	0	0	6	3	0	0	9	0	2
117 " glycerol	9	0	28	0	0	7	0	0	3	8	0	1	7	0	0	1	0	5	3	0	0	9	0	2
118 " maltose	0	0	37	0	0	7	0	0	3	2	2	5	1	0	6	0	0	6	0	0	3	0	0	11
119 " rhamnose	2	0	35	5	0	2	2	0	1	9	0	0	7	0	0	3	0	3	0	0	3	10	0	1
120 " galactose	30	2	5	0	0	7	0	0	3	3	1	5	2	0	5	0	0	6	3	0	0	0	0	11
121 " sucrose	35	0	2	7	0	0	0	0	3	4	0	5	1	0	6	0	0	6	0	0	3	2	0	9
122 " sorbitol	37	0	0	6	1	0	1	2	0	9	0	0	7	0	0	0	1	5	3	0	0	10	0	1
123 " fructose	0	0	37	0	0	7	0	0	3	3	0	6	1	0	6	0	0	6	0	0	3	0	1	10
124 " xylose	26	8	3	0	1	6	1	2	0	9	0	0	5	0	2	5	0	1	0	3	0	8	2	1
125 " trehalose	2	0	35	0	0	7	0	0	3	6	0	3	6	0	1	0	0	6	0	0	3	4	0	7
126 " raffinose	0	0	37	0	0	7	0	0	3	6	2	1	6	0	1	1	2	3	0	0	3	3	0	8
127 " melezitose	15	1	21	0	3	4	0	0	3	6	2	1	6	0	1	0	3	3	2	1	0	10	1	0
128 " cellobiose	34	3	0	0	5	2	1	1	1	5	2	2	1	1	5	2	0	4	0	3	0	5	0	6
129 Litmus milk - acid production	0	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0
130 " " - reduction	2	35	0	0	7	0	0	3	0	6	3	0	4	3	0	1	5	0	3	0	0	8	3	0
131 " " - clot	10	27	0	0	7	0	2	1	0	0	9	0	1	6	0	0	6	0	0	3	0	4	7	0
132 " " - alkaline	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
133 " " - digestion	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
134 Utilization of pyruvate	36	1	0	7	0	0	3	0	0	9	0	0	5	2	0	6	0	0	3	0	0	11	0	0
135 " acetate	36	1	0	7	0	0	3	0	0	9	0	0	7	0	0	5	1	0	3	0	0	11	0	0
136 " propionate	36	1	0	7	0	0	3	0	0	8	1	0	7	0	0	6	0	0	3	0	0	11	0	0
137 " citrate	36	1	0	7	0	0	3	0	0	8	1	0	6	1	0	6	0	0	3	0	0	11	0	0
138 " malate	4	17	16	5	1	1	3	0	0	9	0	0	7	0	0	5	0	1	3	0	0	4	2	5
139 " malonate	37	0	0	6	1	0	3	0	0	8	1	0	6	0	1	4	0	2	3	0	0	11	0	0
140 " -keto-glutarate	36	0	1	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	3	0	0	11	0	0
141 " salicylate	36	0	1	7	0	0	3	0	0	9	0	0	7	0	0	4	0	2	3	0	0	11	0	0
142 " glucose	0	2	35	0	3	4	0	1	2	0	0	9	0	0	7	1	0	5	0	0	3	0	1	10
143 Slime production from sucrose	37	0	0	7	0	0	3	0	0	9	0	0	7	0	0	6	0	0	0	3	0	11	0	0
Number of Strains in each Group	37			7			3			9			7			6			3			11		

Characters	9 *Streptococcus* 2			10 *Streptococcus* 3			11 *Streptococcus* 4			12 *Microbacterium thermosphactum*			13 *Kurthia zopfii*			14 *Erysipelothrix rhusiopathiae*			15 *Gemella haemolysans*			ALL GROUPS		
	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2			
1 Smooth / Rough	8	0	0	4	0	0	5	0	0	25	0	0	5	4	0	11	0	0	4	0	0	137	12	0
2 Entire / Serrated	8	0	0	4	0	0	0	5	0	1	24	0	9	0	0	0	11	0	1	3	0	45	104	0
3 Elevation - low convex	0	8	0	0	4	0	5	0	0	23	2	0	3	6	0	9	2	0	3	1	0	103	46	0
4 - convex	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
5 - fried egg	8	0	0	4	0	0	0	5	0	2	23	0	6	3	0	2	9	0	1	3	0	43	106	0
6 Consistency - watery	0	8	0	0	4	0	4	1	0	12	13	0	9	0	0	5	6	0	2	2	0	48	101	0
7 - butyrous	8	0	0	4	0	0	1	4	0	12	13	0	0	9	0	6	5	0	2	2	0	101	48	0
8 - viscous	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
9 * - bitty	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
10 Translucent colony	2	6	0	1	3	0	0	5	0	21	4	0	9	0	0	2	9	0	3	1	0	48	101	0
11 Homogeneous emulsification	4	4	0	1	3	0	0	5	0	15	10	0	6	3	0	0	11	0	0	4	0	37	112	0
12 Pigmentation - yellow	8	0	0	4	0	0	5	0	0	25	0	0	0	9	0	11	0	0	4	0	0	140	9	0
13 - pink	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
14 Gram positive	0	8	0	0	4	0	0	5	0	0	25	0	1	8	0	0	11	0	4	0	0	6	143	0
15 Cell form - long rods	8	0	0	4	0	0	5	0	0	13	12	0	0	9	0	11	0	0	4	0	0	106	43	0
16 - short rods	8	0	0	4	0	0	5	0	0	11	14	0	1	8	0	0	11	0	4	0	0	94	55	0
17 - coccobacilli	8	0	0	4	0	0	0	5	0	14	11	0	7	2	0	0	11	0	3	1	0	70	79	0
18 - cocci	0	8	0	0	4	0	0	5	0	25	0	0	9	0	0	11	0	0	0	4	0	114	35	0
19 - filaments	8	0	0	4	0	0	5	0	0	12	13	0	5	4	0	11	0	0	4	0	0	113	36	0
20 - chains	0	8	0	0	4	0	0	5	0	2	23	0	0	9	0	0	11	0	0	4	0	3	146	0
21 Adherence	3	5	0	4	0	0	1	4	0	23	2	0	7	2	0	0	11	0	4	0	0	82	67	0
22 Haemolysis - alpha	1	7	0	3	1	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	140	9	0
23 - beta	7	1	0	2	2	0	0	5	0	25	0	0	9	0	0	11	0	0	4	0	0	98	51	0
24 Colony diameter <1/1-3/>3mm	2	6	0	1	3	0	0	5	0	2	19	4	0	0	9	9	2	0	1	3	0	53	83	13
25 Pleomorphism	8	0	0	4	0	0	5	0	0	10	15	0	9	0	0	11	0	0	3	1	0	127	22	0
26 Rhizoidal margin	0	8	0	0	4	0	4	1	0	25	0	0	0	9	0	11	0	0	4	0	0	119	30	0
27 Pellet - stringy	0	8	0	0	4	0	0	5	0	1	24	0	1	8	0	0	11	0	2	2	0	6	143	0
28 - granular	8	0	0	4	0	0	5	0	0	25	0	0	8	1	0	11	0	0	2	2	0	145	4	0
29 - floculent	8	0	0	4	0	0	5	0	0	24	1	0	9	0	0	7	4	0	4	0	0	143	6	0
30 Pellicle? Ring/ neither	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
31 Easy pellet resuspension	1	7	0	1	3	0	0	5	0	12	13	0	4	5	0	8	3	0	0	4	0	32	117	0
32 Beaded Gram stain	8	0	0	4	0	0	5	0	0	0	25	0	7	2	0	11	0	0	4	0	0	97	52	0
33 Motile	0	8	0	0	4	0	5	0	0	25	0	0	3	6	0	11	0	0	4	0	0	78	71	0
34 Tumbling	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	111	38	0
35 Cellulose hydrolysis	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
36 Tween 20 "	8	0	0	4	0	0	5	0	0	25	0	0	1	8	0	11	0	0	4	0	0	141	8	0
37 Tween 40 "	8	0	0	4	0	0	4	1	0	23	2	0	7	2	0	11	0	0	4	0	0	140	9	0
38 Tween 60 "	3	5	0	1	3	0	5	0	0	2	23	0	7	2	0	11	0	0	0	4	0	53	96	0
39 Tween 80 "	8	0	0	4	0	0	4	1	0	25	0	0	0	9	0	11	0	0	4	0	0	138	11	0
40 Tyrosine "	4	4	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	144	5	0
41 Xanthine "	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
42 Growth at 5°C	0	2	6	2	1	1	2	3	0	0	0	25	1	2	6	5	4	2	4	0	0	26	27	96
43 " 10°C	0	1	7	0	3	1	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	4	24	121
44 " 20°C	0	0	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149
45 " 30°C	0	0	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149
46 " 35°C	0	0	8	0	0	4	0	0	5	9	16	0	0	0	9	0	0	11	0	0	4	9	16	124
47 " 45°c	1	0	7	0	3	1	0	5	0	0	25	0	0	5	4	0	11	0	0	4	0	3	88	58
48 " 50°C	7	0	1	4	0	0	4	1	0	24	1	0	9	0	0	11	0	0	4	0	0	138	6	5
49 Gluconate oxidation	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	147	2	0
50 Indole from tryptone	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
51 Lecithinase activity	1	7	0	4	0	0	0	5	0	12	13	0	0	9	0	2	9	0	0	4	0	35	114	0
52 Methyl Red Test	0	8	0	0	4	0	0	5	0	0	25	0	9	0	0	0	11	0	0	4	0	9	140	0
53 Voges-Proskauer Test	2	6	0	3	1	0	1	4	0	3	11	11	9	0	0	11	0	0	2	0	2	59	74	16
54 Innib'n by Thallous acetate 0.01%	8	0	0	4	0	0	5	0	0	25	0	0	0	9	0	11	0	0	4	0	0	140	9	0
55 " 0.02%	8	0	0	4	0	0	5	0	0	25	0	0	0	8	1	11	0	0	4	0	0	140	8	1
56 " 0.03%	8	0	0	4	0	0	5	0	0	25	0	0	0	8	1	11	0	0	4	0	0	140	8	1
57 Catalase activity	6	2	0	3	1	0	5	0	0	4	21	0	0	9	0	11	0	0	4	0	0	69	80	0
58 Oxidase activity	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
59 Growth under hydrogen	0	8	0	0	4	0	0	5	0	0	25	0	9	0	0	0	11	0	0	4	0	10	139	0
60 Inhib'n by sodium chloride 6.5%	6	2	0	0	2	2	0	5	0	13	12	0	4	5	0	2	7	2	0	4	0	79	54	16
61 " 10 %	3	5	0	0	2	2	0	4	1	3	20	2	1	8	0	1	6	4	0	4	0	27	90	32
62 Red'n of potassium tellurite 0.01 %	0	8	0	4	0	0	4	1	0	1	24	0	0	9	0	1	10	0	4	0	0	22	127	0
63 " " 0.025%	0	8	0	4	0	0	5	0	0	8	17	0	0	9	0	1	10	0	4	0	0	37	112	0
64 " " 0.05 %	2	6	0	3	1	0	0	5	0	3	22	0	2	7	0	3	8	0	4	0	0	34	115	0
65 Inhib'n by tetrazollium 0.01%	8	0	0	4	0	0	5	0	0	20	5	0	9	0	0	11	0	0	4	0	0	137	12	0
66 Red'n of " 0.01%	0	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	6	143	0
67 Inhib'n by " 0.1 %	7	0	1	1	0	3	0	0	5	0	12	13	0	8	1	11	0	0	4	0	0	71	46	32
68 Red'n of " 0.1 %	1	7	0	3	1	0	5	0	0	17	8	0	1	8	0	0	11	0	0	4	0	43	106	0
69 Inhib'n by pot. thiocyanate 2.5%	0	7	1	0	0	4	0	0	5	0	21	4	0	9	0	0	0	11	0	4	0	47	65	37
70 " " 3.7%	0	7	1	0	0	4	0	0	5	0	0	25	0	9	0	0	0	11	0	4	0	47	39	63
71 " " 4.5%	0	4	4	0	0	4	0	0	5	0	0	25	0	6	3	0	0	11	0	4	0	47	28	74
72 " sodium azide 0.01%	8	0	0	1	2	1	0	1	4	0	1	24	0	0	9	7	3	1	0	4	0	48	30	71
73 " " 0.02%	7	1	0	0	1	3	0	0	5	0	0	25	0	0	9	0	10	1	0	4	0	28	39	82
74 " " 0.05%	8	0	0	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	4	0	17	22	110
75 Ammonia from peptone	8	0	0	4	0	0	5	0	0	25	0	0	0	9	0	11	0	0	4	0	0	140	9	0
76 Hippurate hydrolysis	2	6	0	3	1	0	4	1	0	25	0	0	3	6	0	5	6	0	4	0	0	78	71	0
77 Arginine deamination (+oxygen)	8	0	0	4	0	0	5	0	0	25	0	0	0	9	0	11	0	0	4	0	0	139	10	0
78 " (-oxygen)	2	6	0	1	3	0	0	5	0	25	0	0	9	0	0	0	11	0	4	0	0	108	41	0
79 Aesculin hydrolysis	0	8	0	1	3	0	0	5	0	0	25	0	9	0	0	11	0	0	4	0	0	44	105	0
80 Sulphatase activity	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
81 Phosphatase activity	2	6	0	3	1	0	5	0	0	0	25	0	0	9	0	5	6	0	0	4	0	23	126	0
82 DNase "	0	8	0	2	2	0	5	0	0	0	25	0	5	4	0	11	0	0	0	4	0	31	118	0
83 RNase "	2	6	0	3	1	0	5	0	0	0	25	0	7	2	0	11	0	0	0	4	0	40	109	0
84 Gas from glucose	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	143	6	0
85 Urease activity	8	0	0	4	0	0	5	0	0	25	0	0	0	9	0	11	0	0	4	0	0	139	10	0
86 Hydrogen sulphide production	6	2	0	3	1	0	5	0	0	25	0	0	9	0	0	4	7	0	1	3	0	110	39	0
87 Inhib'n by Penicillin	1	7	0	0	3	1	0	2	3	0	25	0	0	5	4	0	11	0	0	4	0	2	88	59
88 " Streptomycin	0	8	0	0	2	2	0	2	3	24	1	0	0	7	2	0	11	0	0	4	0	25	74	50
89 " Chloramphenicol	0	8	0	0	3	1	0	2	3	0	25	0	0	8	1	0	11	0	0	4	0	0	92	57
90 " Chlortetracycline	0	8	0	0	2	2	0	2	3	0	25	0	0	7	2	0	11	0	0	4	0	0	86	63
91 " Oxytetracycline	0	8	0	0	2	2	0	3	2	0	25	0	0	8	1	0	11	0	0	4	0	0	98	51
92 " Erythromycin	0	8	0	0	3	1	0	2	3	0	25	0	0	9	0	0	11	0	0	4	0	0	94	55
93 " Tetracycline	0	8	0	0	3	1	0	2	3	0	25	0	0	8	1	0	11	0	0	4	0	0	98	51
94 " Sulphanilamide	8	0	0	3	1	0	2	1	2	9	16	0	7	2	0	8	3	0	1	3	0	114	33	2
95 " Novobiocin	0	8	0	0	3	1	0	4	1	0	25	0	0	9	0	11	0	0	0	4	0	12	104	33
96 " Oleandomycin	0	8	0	0	3	1	0	4	1	0	25	0	0	9	0	0	11	0	0	4	0	0	99	50
97 " Naladixic acid	6	2	0	4	0	0	5	0	0	0	25	0	1	8	0	2	8	1	2	2	0	102	46	1
98 Stimul'n by Sulphanilamide	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	118	31	0
99 Survival at 60°C for 15 min.	0	8	0	2	2	0	3	2	0	24	1	0	1	8	0	10	1	0	4	0	0	89	60	0
100 Nitrate red'n to nitrite	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	146	3	0
101 " " nitrogen	8	0	0	4	0	0	5	0	0	25	0	0	8	1	0	11	0	0	4	0	0	148	1	0
102 Growth in the presence of nitrite	8	0	0	[illegible]	2	[illegible]	[illegible]	5	[illegible]	25	[illegible]	[illegible]	1	8	[illegible]	11	0	0	4	0	0	[illegible]	[illegible]	[illegible]
103 Starch hydrolysis	7	1	0	[illegible]	1	0	[illegible]	5	0	[illegible]	6	0	[illegible]	0	0	11	0	0	4	0	0	101	48	0
104 Casein "	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
105 Glucose breakdown (+oxygen)	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
106 " " (-oxygen)	0	8	0	0	4	0	0	5	0	0	25	0	9	0	0	0	11	0	0	4	0	9	140	0
107 Acid from inulin	0	6	2	2	0	2	1	0	4	1	23	1	9	0	0	11	0	0	4	0	0	89	46	14
108 " inositol	3	3	2	4	0	0	5	0	0	0	0	25	9	0	0	11	0	0	4	0	0	99	21	29
109 " dulcitol	2	6	0	4	0	0	5	0	0	1	23	1	9	0	0	11	0	0	4	0	0	98	49	2
110 " lactose	0	0	8	0	0	4	0	0	5	0	0	25	9	0	0	0	0	11	4	0	0	20	10	119
111 " -methyl-glucoside	0	5	3	4	0	0	0	4	1	0	0	25	9	0	0	11	0	0	4	0	0	44	28	77
112 " melibiose	0	7	1	4	0	0	0	5	0	0	0	25	9	0	0	11	0	0	4	0	0	79	24	46
113 " salicin	0	0	8	3	0	1	0	4	1	0	1	24	9	0	0	11	0	0	4	0	0	43	6	100
114 " adonitol	0	7	1	4	0	0	5	0	0	1	24	0	9	0	0	11	0	0	4	0	0	81	67	1
115 " arabinose	4	0	4	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	137	2	10
116 " mannitol	2	0	6	3	0	1	5	0	0	3	0	22	9	0	0	11	0	0	4	0	0	98	0	51
117 " glycerol	3	0	5	4	0	0	5	0	0	0	0	25	3	0	6	11	0	0	4	0	0	67	0	82
118 " maltose	0	0	8	0	0	4	0	0	5	0	0	25	9	0	0	0	8	3	0	0	4	12	10	127
119 " rhamnose	4	0	4	3	0	1	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	99	0	50
120 " galactose	0	0	8	2	0	2	0	0	5	0	1	24	9	0	0	0	0	11	4	0	0	53	4	92
121 " sucrose	0	0	8	1	0	3	0	0	5	0	0	25	9	0	0	8	0	3	0	0	4	67	0	82
122 " sorbitol	3	1	4	3	0	1	5	0	0	0	0	25	9	0	0	11	0	0	4	0	0	108	5	36
123 " fructose	0	0	8	1	0	3	0	0	5	0	0	25	7	0	2	0	0	11	0	0	4	12	1	136
124 " xylose	3	2	3	2	1	1	1	0	4	1	0	24	9	0	0	10	0	1	2	0	2	82	19	48
125 " trehalose	1	0	7	2	0	2	0	0	5	0	0	25	9	0	0	9	0	2	4	0	0	43	0	106
126 " raffinose	0	0	8	2	0	2	0	0	5	0	0	25	9	0	0	1	3	7	4	0	0	32	7	110
127 " melezitose	1	2	5	4	0	0	1	4	0	0	0	25	9	0	0	11	0	0	4	0	0	69	17	63
128 " cellobiose	0	1	7	2	1	1	0	5	0	0	0	25	9	0	0	11	0	0	4	0	0	74	22	53
129 Litmus milk - acid production	0	8	0	2	2	0	0	5	0	3	22	0	9	0	0	0	11	0	0	4	0	14	135	0
130 " " - reduction	3	5	0	3	1	0	5	0	0	25	0	0	3	6	0	11	0	0	4	0	0	78	71	0
131 " " - clot	0	8	0	3	1	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	74	75	0
132 " " - alkaline	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	149	0	0
133 " " - digestion	8	0	0	4	0	0	5	0	0	25	0	0	8	1	0	11	0	0	4	0	0	148	1	0
134 Utilization of pyruvate	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	146	3	0
135 " acetate	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	147	2	0
136 " propionate	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	147	2	0
137 " citrate	8	0	0	3	1	0	5	0	0	25	0	0	8	0	1	11	0	0	4	0	0	144	4	1
138 " malate	1	4	3	1	2	1	4	1	0	25	0	0	7	2	0	10	1	0	0	1	3	88	31	30
139 " malonate	8	0	0	4	0	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	144	2	3
140 " -keto-glutarate	8	0	0	3	1	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	147	1	1
141 " salicylate	4	0	4	4	0	0	5	0	0	25	0	0	8	1	0	9	2	0	4	0	0	139	3	7
142 " glucose	0	0	8	0	0	4	0	0	5	6	18	1	5	4	0	0	1	10	0	0	4	12	30	107
143 Slime production from sucrose	8	0	0	3	1	0	5	0	0	25	0	0	9	0	0	11	0	0	4	0	0	145	4	0
Number of Strains in each Group	8			4			5			25			9			11			4			149		

APPENDIX IV.

THE CODED RESULTS FROM THE NUMERICAL TAXONOMIC SURVEY.

TABLE I. Test Results for all Strains.

Strain No.	Character Nos.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
G 1	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 2	0	0	1	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 3	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 4	0	0	1	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 5	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 6	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 7	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 8	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 9	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0	0
G 10	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 11	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 12	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 13	0	1	1	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 14	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 15	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 15a	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 16	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 16a	1	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 17	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 18	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 19	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 20	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 21	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 22	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 23	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 24	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 25	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 26	1	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 27	0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0
G 28	0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0
G 29	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 30	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 31	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 32	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 33	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 34	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 35	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 36	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 37	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 38	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 39	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 40	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0
G 42	0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0
G 43	0	1	1	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 44	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	1	0
G 45	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	1	0
G 46	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	1
G 47	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	1	1
G 48	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	1
G 60	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	1	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
G 1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0
G 2	0	1	1	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0
G 3	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0
G 4	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 5	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0
G 6	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 7	0	1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0
G 8	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 9	0	1	0	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0
G 10	1	1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0
G 11	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 12	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 13	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 14	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 15	0	1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0
G 15a	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 16	0	1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0
G 16a	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 17	0	1	1	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0
G 18	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 19	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 20	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 21	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 22	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 23	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
G 24	0	1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0
G 25	0	1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0
G 26	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 27	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 28	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 29	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 30	1	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 31	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 32	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 33	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 34	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 35	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 36	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 37	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 38	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 39	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 40	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0
G 42	0	1	1	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0
G 43	0	1	1	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0
G 44	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 45	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 46	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 47	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 48	0	1	1	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0
G 60	1	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
G 1	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 2	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 3	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 4	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 5	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 6	0	1	0	0	0	2	2	2	2	2	2	0	0	0	0	1	1	0
G 7	0	1	0	0	0	2	2	2	2	2	2	0	0	0	0	1	1	0
G 8	0	0	0	0	0	2	2	2	2	2	1	0	0	0	0	1	1	0
G 9	0	1	0	0	0	2	2	2	2	2	2	1	0	0	1	1	1	0
G 10	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 11	0	0	0	0	0	2	2	2	2	2	1	0	0	0	0	1	1	0
G 12	0	1	0	0	0	2	0	2	2	2	2	0	0	0	1	1	1	0
G 13	0	1	0	0	0	2	2	2	2	2	2	0	0	0	0	1	1	0
G 14	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 15	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 15a	0	0	0	0	0	2	2	2	2	2	1	0	0	0	0	1	1	0
G 16	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 16a	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 17	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 18	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 19	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 20	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 21	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 22	0	1	0	0	0	2	2	2	2	2	1	0	0	0	0	1	1	0
G 23	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 24	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 25	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 26	0	1	0	0	0	2	2	2	2	2	2	2	0	0	1	1	1	0
G 27	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 28	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 29	0	1	0	0	0	1	2	2	2	2	1	0	0	0	1	1	1	0
G 30	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 31	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	2	0
G 32	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 33	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 34	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 35	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 36	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 37	0	1	0	0	0	2	2	2	2	2	2	0	0	0	0	1	1	0
G 38	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 39	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 40	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 42	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 43	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	0	0
G 44	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 45	0	0	0	0	0	2	2	2	2	2	2	0	0	0	0	1	1	0
G 46	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 47	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 48	0	0	0	0	0	0	1	2	2	2	1	0	0	0	1	1	0	0
G 60	0	0	1	0	0	0	1	2	2	2	2	0	0	0	1	1	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
G 1	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 2	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 3	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 4	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 5	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 6	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 7	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 8	0	0	0	0	1	0	1	1	1	1	0	1	0	1	0	0	0	1
G 9	0	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	2	0
G 10	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 11	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 12	0	0	1	0	0	0	1	1	1	1	0	1	0	1	0	0	0	2
G 13	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 14	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 15	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 15a	0	0	0	0	1	1	1	1	1	1	0	1	0	1	0	0	0	2
G 16	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 16a	0	0	0	0	1	1	1	1	1	1	0	1	0	1	0	0	0	1
G 17	0	0	1	0	1	0	0	1	1	1	0	1	2	1	0	0	0	2
G 18	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 19	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	1
G 20	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	1
G 21	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 22	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 23	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 24	0	0	1	0	1	1	2	1	1	1	0	1	0	1	0	0	0	2
G 25	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 26	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	1
G 27	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 28	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 29	0	0	1	0	1	1	1	1	1	1	0	1	0	1	0	0	0	2
G 30	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 31	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 32	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 33	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 34	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 35	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	2
G 36	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 37	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 38	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 39	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 40	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	2
G 42	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	0
G 43	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	0	0	2
G 44	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 45	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 46	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 47	0	0	1	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 48	0	0	0	0	1	2	2	1	1	1	0	1	1	1	1	2	2	0
G 60	0	0	0	0	1	2	2	0	0	0	0	0	2	0	2	2	2	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
G 1	2	2	0	1	0	0	1	0	1	1	1	0	0	0	1	2	2	2
G 2	1	2	0	0	0	0	1	0	1	1	1	0	0	0	2	1	2	2
G 3	1	2	0	0	0	0	1	0	1	1	1	0	0	0	2	1	2	2
G 4	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 5	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 6	2	2	0	1	0	0	1	0	1	1	1	0	0	0	1	1	2	2
G 7	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 8	2	2	0	1	0	0	1	0	1	1	1	0	1	0	1	1	1	2
G 9	0	0	0	1	0	0	1	0	1	0	1	0	0	1	2	2	2	2
G 10	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 11	2	2	0	1	0	0	1	0	1	1	1	0	0	0	1	2	1	2
G 12	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 13	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	1	2	2
G 14	2	2	0	1	0	0	1	0	1	1	1	0	0	0	1	2	2	2
G 15	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 15a	2	2	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
G 16	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 16a	1	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 17	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 18	2	2	0	1	0	0	1	0	1	1	1	0	0	0	1	2	2	2
G 19	1	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 20	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	1	2	2
G 21	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 22	2	2	0	1	0	0	1	0	1	1	1	0	0	0	1	1	2	2
G 23	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 24	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 25	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 26	1	1	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 27	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 28	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 29	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 30	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 31	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 32	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 33	2	2	0	1	1	0	1	0	1	1	1	0	0	0	2	2	2	2
G 34	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 35	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 36	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 37	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 38	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 39	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 40	2	2	0	1	0	0	1	0	1	1	1	0	0	0	2	1	2	2
G 42	1	1	0	0	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 43	2	2	0	1	0	0	1	0	1	1	0	0	0	0	2	2	2	2
G 44	1	2	0	1	0	0	1	0	1	1	1	0	0	1	2	1	2	2
G 45	1	2	0	1	0	0	1	0	1	1	1	0	0	1	2	1	2	2
G 46	1	2	0	1	0	0	1	0	1	1	1	0	0	1	2	1	2	2
G 47	1	2	0	0	0	0	1	0	1	1	1	0	0	1	2	1	2	2
G 48	0	1	0	1	0	1	0	0	1	1	1	0	0	1	2	2	2	2
G 60	0	1	0	0	0	0	1	0	1	1	1	0	0	1	2	2	2	2

TABLE I. (continued).

Strain No.	Character Nos.																	
	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
G 1	1	2	1	0	1	1	0	1	0	0	0	1	0	0	0	1	0	0
G 2	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	1	1
G 3	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	1	1
G 4	2	2	2	0	1	2	0	1	0	0	0	1	0	0	0	1	0	0
G 5	2	2	1	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 6	2	2	2	0	1	2	0	1	0	0	0	1	1	0	0	1	0	0
G 7	2	2	1	0	1	2	0	0	0	0	0	1	0	0	0	1	0	0
G 8	1	1	2	0	1	1	0	0	0	0	0	1	0	0	0	1	2	2
G 9	2	2	2	0	1	1	0	0	0	0	0	0	0	0	0	1	2	0
G 10	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 11	1	2	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	1
G 12	1	2	2	0	1	2	0	1	0	0	0	1	0	0	0	1	0	0
G 13	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 14	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 15	2	2	2	0	1	2	0	1	0	0	0	1	0	0	0	1	0	0
G 15a	1	1	1	0	0	2	0	0	0	0	0	1	0	0	0	1	2	1
G 16	2	2	2	0	2	2	0	0	0	0	0	1	0	0	0	1	0	0
G 16a	2	2	2	0	1	2	0	0	0	0	0	1	0	0	0	1	2	1
G 17	2	2	2	0	1	2	0	1	0	0	0	1	0	0	0	1	0	0
G 18	2	2	1	0	1	1	0	1	0	0	0	1	0	0	0	1	0	0
G 19	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 20	2	2	2	0	1	1	0	1	0	0	0	1	0	0	0	1	0	0
G 21	2	2	2	1	2	2	0	0	0	0	0	1	0	0	0	1	0	0
G 22	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 23	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 24	2	2	2	0	2	2	0	1	0	0	0	1	1	0	0	1	0	0
G 25	2	2	2	0	1	2	0	1	1	0	0	1	0	0	0	1	0	0
G 26	2	2	2	0	2	2	0	1	1	0	0	1	0	0	0	1	0	0
G 27	2	2	2	0	2	2	0	1	1	0	0	1	0	0	0	1	0	0
G 28	2	2	2	0	2	2	0	0	1	0	0	1	0	0	0	1	0	0
G 29	2	2	2	0	2	2	0	0	1	0	0	1	0	0	0	1	0	1
G 30	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	1
G 31	2	2	2	0	2	2	0	0	1	0	0	0	0	0	0	1	0	1
G 32	2	2	2	0	2	2	0	1	0	0	0	1	0	0	0	1	0	0
G 33	2	2	2	0	2	2	0	0	1	0	0	1	0	0	0	1	0	0
G 34	2	2	2	0	1	2	0	1	1	0	0	1	0	0	0	1	0	0
G 35	2	1	2	0	2	2	0	1	1	0	0	1	0	0	0	1	0	0
G 36	2	2	2	0	1	2	0	0	0	0	0	1	0	0	0	1	0	0
G 37	2	2	2	0	2	2	0	0	0	0	0	1	0	0	0	1	0	0
G 38	2	2	2	0	2	2	0	0	1	0	0	1	0	0	0	1	0	0
G 39	2	2	2	0	1	2	0	1	0	0	0	1	0	0	0	1	0	0
G 40	2	2	2	0	2	2	0	0	1	0	0	1	1	0	0	1	0	0
G 42	2	2	2	0	2	2	0	0	1	0	0	1	1	0	0	1	0	1
G 43	2	2	2	0	2	2	0	0	1	1	0	1	0	1	0	1	0	1
G 44	2	2	2	0	2	2	0	0	0	1	0	1	1	0	0	1	0	0
G 45	2	2	2	0	2	2	0	0	1	1	0	1	1	0	0	1	0	1
G 46	2	2	2	0	2	2	0	0	1	1	0	1	1	0	0	1	0	1
G 47	2	2	2	0	2	2	0	1	0	0	0	0	1	0	0	1	1	0
G 48	2	2	2	2	2	2	0	0	0	0	0	0	1	0	0	1	1	0
G 60	2	2	1	0	1	2	0	0	0	0	0	0	1	0	0	1	2	0

TABLE I. (continued).

Strain No.	Character Nos.																		
	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
G 1	1	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 2	0	2	1	2	2	1	0	2	2	2	0	2	0	0	2	1	2	2
G 3	0	2	1	2	2	1	0	2	2	2	0	2	0	0	2	2	2	2
G 4	0	2	2	2	2	0	0	0	2	2	2	0	0	0	2	1	2	2
G 5	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 6	0	2	2	0	2	0	0	0	0	2	2	0	0	0	2	1	2	2
G 7	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 8	2	2	2	0	2	0	0	2	2	2	2	2	2	0	2	0	2	2
G 9	0	2	2	2	2	1	0	2	2	2	0	2	2	2	2	1	2	2
G 10	0	1	2	2	2	0	0	0	0	2	2	0	0	0	2	1	2	2
G 11	0	1	2	0	2	0	0	0	0	2	2	0	0	0	2	1	2	2
G 12	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 13	0	2	2	0	2	0	0	0	2	2	2	2	2	0	2	0	2	2
G 14	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 15	0	0	2	0	2	0	0	0	0	2	0	0	0	0	2	2	0	2
G 15a	1	2	2	0	2	0	1	2	2	2	0	2	2	1	2	1	2	2
G 16	0	0	2	0	2	0	0	0	0	2	0	0	2	0	2	2	0	2
G 16a	0	2	2	1	2	1	0	2	2	2	0	2	2	1	2	1	2	2
G 17	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 18	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 19	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 20	0	2	2	0	2	0	0	0	2	2	2	1	0	0	2	1	2	2
G 21	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 22	0	2	2	0	2	0	0	0	2	2	2	1	0	0	2	1	2	2
G 23	0	2	2	0	2	1	0	0	2	2	2	0	0	0	2	0	2	2
G 24	0	2	2	0	2	1	0	0	2	2	2	0	0	0	2	0	2	2
G 25	0	2	2	0	2	1	0	0	2	2	2	0	0	0	2	0	2	2
G 26	1	2	2	2	2	1	0	0	2	2	2	0	0	0	2	1	2	2
G 27	0	1	1	0	2	1	0	0	2	2	2	0	0	0	2	0	2	2
G 28	0	2	2	0	2	1	0	0	2	2	2	0	0	0	2	0	2	2
G 29	0	2	2	0	2	1	0	0	2	2	2	2	0	0	2	0	2	2
G 30	0	2	2	0	2	1	0	2	2	2	2	2	0	0	2	0	2	2
G 31	0	2	2	0	2	1	0	0	2	2	2	2	0	0	2	2	2	2
G 32	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	1	2	2
G 33	0	2	2	0	2	1	0	0	2	2	2	0	0	0	2	0	2	2
G 34	0	1	2	0	2	1	0	0	0	2	2	0	0	0	2	0	2	2
G 35	0	1	2	0	2	1	0	2	2	2	2	2	0	0	2	0	2	2
G 36	0	2	2	0	2	1	0	0	0	2	2	0	0	0	2	0	2	2
G 37	0	2	2	0	2	1	0	0	0	2	2	0	0	0	2	0	2	2
G 38	0	2	2	0	2	1	0	0	2	2	2	0	0	0	2	0	2	2
G 39	0	2	2	0	2	1	0	0	0	2	2	0	0	0	2	0	2	2
G 40	0	2	2	0	2	1	0	2	2	2	2	0	0	0	2	0	2	2
G 42	1	2	1	0	2	1	0	2	2	2	0	2	0	0	2	2	2	2
G 43	1	2	2	2	2	1	2	0	2	2	0	2	0	0	2	2	2	2
G 44	1	2	1	2	2	1	0	2	2	2	2	2	0	0	2	2	2	2
G 45	1	2	1	2	2	1	0	2	2	2	2	2	0	0	2	2	2	2
G 46	1	2	1	2	2	1	0	2	2	2	0	2	0	0	2	2	2	2
G 47	1	2	1	2	2	1	1	2	2	2	0	2	0	1	2	2	2	2
G 48	1	2	2	2	2	1	1	0	2	2	0	2	2	1	2	2	2	2
G 60	1	2	1	0	2	0	0	0	0	2	0	2	2	0	2	0	2	2

TABLE I. (continued).

Strain No.	Character Nos.																
	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
G 1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 2	2	2	1	1	1	0	0	0	0	0	0	1	1	0	0	2	0
G 3	2	2	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
G 4	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 5	2	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 6	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 7	2	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 8	2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
G 9	2	1	1	1	1	0	0	0	0	0	0	1	0	0	2	2	0
G 10	2	1	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 11	2	1	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 12	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0
G 13	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 14	0	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 15	0	0	1	1	0	0	0	0	0	1	0	2	0	2	0	2	0
G 15a	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	0
G 16	2	0	1	1	0	0	0	0	0	0	0	1	0	0	2	2	0
G 16a	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	2	0
G 17	2	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 18	0	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 19	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 20	2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
G 21	2	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 22	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 23	0	0	1	1	0	0	0	1	0	0	1	2	0	0	0	2	0
G 24	0	0	1	1	0	0	0	0	0	0	0	2	0	0	0	2	0
G 25	2	0	1	1	0	0	0	0	0	0	0	1	0	0	0	2	0
G 26	2	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 27	0	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 28	2	0	1	1	0	0	0	0	0	0	0	2	0	0	0	2	0
G 29	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0
G 30	2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 31	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	2	0
G 32	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 33	0	0	1	1	0	0	0	0	0	0	0	2	0	0	0	2	0
G 34	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 35	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 36	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 37	2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 38	0	0	1	1	1	0	0	0	1	0	0	1	0	0	0	2	0
G 39	2	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 40	0	1	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 42	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 43	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 44	1	1	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 45	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
G 46	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
G 47	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 48	2	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
G 60	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
G 61	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0
G 62	0	1	0	0	1	0	1	0	0	1	0	0	0	1	1	1	0	0
G 63	0	0	1	0	0	1	0	0	0	1	1	0	0	1	1	1	1	0
G 64	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	1	0	0
G 65	0	0	1	0	0	1	0	0	0	1	1	0	0	1	1	1	0	0
G 66	1	1	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0
G 67	0	1	0	0	1	1	0	0	0	1	1	0	0	1	1	1	0	0
G 68	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	0
G 69	0	1	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0
G 70	1	1	0	0	1	1	0	0	0	1	1	0	0	1	1	1	0	0
G 71	0	0	1	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0
G 72	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	0
G 73	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	0
G 74	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	0
G 75	1	1	0	0	1	1	0	0	0	1	1	0	0	1	1	1	0	0
G 76	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	0
G 77	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	0	0
G 78	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	0	0
G 79	0	1	0	0	1	1	0	0	0	1	1	0	0	1	1	1	0	0
G 80	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	0
G 81	0	1	0	0	1	0	1	0	0	1	0	0	0	1	1	0	0	0
G 82	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1
G 83	0	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1
G 84	0	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1
G 85	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1
G 86	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0
G 87	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0
G 88	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0
G 89	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0
G 90	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0
G 91	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0
G 92	0	1	0	0	1	0	1	0	0	1	0	0	0	1	1	0	0	0
G 93	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0
G 94	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0
G 95	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0
G 96	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	1	0
G 97	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	1	0
G 98	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	1	0
G 99	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	1	0	0
G 100	0	1	0	0	1	1	0	0	0	0	1	0	0	1	1	0	0	0
G 101	0	1	0	0	1	1	0	0	0	0	1	0	0	1	1	1	1	0
G 102	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0
G 103	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0
G 104	0	1	0	0	1	1	0	0	0	0	1	0	0	1	1	0	0	0
G 105	0	1	0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0
G 106	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0
G 107	0	1	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0
G 108	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	1	0
G 109	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0
G 110	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
G 61	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 62	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
G 63	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 64	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 65	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 66	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 67	1	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
G 68	1	1	0	0	0	0	1	0	1	0	0	0	1	1	1	0	0	0
G 69	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 70	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 71	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 72	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 73	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 74	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 75	0	1	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 76	1	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
G 77	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 78	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
G 79	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0
G 80	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 81	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
G 82	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0
G 83	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 84	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 85	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0
G 86	0	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0
G 87	0	1	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0
G 88	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0
G 89	1	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0
G 90	1	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0
G 91	1	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0
G 92	1	1	0	0	0	2	1	0	1	0	0	0	0	1	0	0	0	0
G 93	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
G 94	1	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0
G 95	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0
G 96	0	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
G 97	0	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0
G 98	0	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
G 99	1	1	1	0	0	2	0	0	1	0	0	0	1	1	0	0	0	0
G 100	1	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
G 101	0	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
G 102	0	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0
G 103	1	1	0	0	0	2	1	0	1	0	0	0	0	1	0	0	0	0
G 104	1	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
G 105	1	1	0	0	0	2	0	0	1	0	0	0	0	1	0	0	0	0
G 106	0	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0
G 107	1	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
G 108	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
G 109	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0
G 110	0	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
G 61	0	0	0	0	0	0	1	2	2	2	2	0	0	0	1	1	1	0
G 62	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	0	0
G 63	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	0	0
G 64	0	0	0	0	0	1	1	2	2	2	2	0	0	0	1	1	0	0
G 65	0	1	0	0	0	2	2	2	2	2	2	0	1	0	1	1	1	0
G 66	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 67	0	1	0	0	0	1	1	2	2	2	1	0	0	0	1	1	0	0
G 68	1	1	0	0	0	1	1	2	2	2	2	2	0	0	1	1	0	0
G 69	0	1	0	0	0	1	1	2	2	2	2	1	1	0	1	1	0	0
G 70	0	1	0	0	0	1	1	2	2	2	2	1	0	0	0	1	0	0
G 71	1	1	0	0	0	1	2	2	2	2	2	1	0	0	0	1	0	0
G 72	0	0	0	0	0	0	2	2	2	2	2	1	0	0	1	1	0	0
G 73	0	1	0	0	0	2	1	2	2	2	1	0	0	0	1	1	0	0
G 74	0	0	0	0	0	0	1	2	2	2	0	0	0	0	1	1	0	0
G 75	0	0	0	0	0	0	1	2	2	2	2	0	0	0	1	1	2	0
G 76	0	0	0	0	0	1	1	2	2	2	2	0	0	0	0	1	0	0
G 77	0	1	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 78	0	1	0	0	0	0	1	2	2	2	2	0	0	0	1	1	1	0
G 79	0	1	0	0	0	1	0	2	2	2	2	0	0	0	1	1	1	0
G 80	0	0	0	0	0	1	0	2	2	2	2	0	0	0	0	1	0	0
G 81	0	1	0	0	0	0	0	2	2	2	2	2	0	0	1	1	0	0
G 82	0	1	0	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 83	0	1	0	0	0	0	2	2	2	2	1	0	0	0	1	1	2	0
G 84	0	1	0	0	0	0	2	2	2	2	1	0	0	0	1	1	2	0
G 85	0	1	0	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 86	0	1	0	0	0	2	2	2	2	0	1	0	0	0	1	1	2	0
G 87	0	1	0	0	0	2	2	2	2	1	1	0	0	0	0	1	1	0
G 88	0	0	0	0	0	2	2	2	2	1	1	0	0	0	0	1	2	0
G 89	0	1	0	0	0	2	2	2	2	0	1	0	0	0	0	1	1	0
G 90	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	2	0
G 91	0	0	0	0	0	2	2	2	2	0	1	0	0	0	0	1	1	0
G 92	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	2	0
G 93	1	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	2	0
G 94	0	1	0	0	0	2	2	2	2	1	1	0	0	0	0	1	1	0
G 95	0	1	0	0	0	2	2	2	2	0	1	0	0	0	1	1	1	0
G 96	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	2	0
G 97	0	1	0	0	0	2	2	2	2	1	1	0	0	0	0	1	2	0
G 98	0	1	0	0	0	2	2	2	2	1	1	0	0	0	0	1	1	0
G 99	0	1	0	0	0	2	2	2	2	0	1	0	0	0	1	1	0	0
G 100	0	1	0	0	0	2	2	2	2	0	1	0	0	0	1	1	0	0
G 101	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	1	0
G 102	0	1	0	0	0	2	2	2	2	1	1	1	0	0	0	1	2	0
G 103	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	1	0
G 104	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	2	0
G 105	1	1	0	0	0	2	2	2	2	0	1	0	0	0	0	1	1	0
G 106	0	1	0	0	0	2	2	2	2	0	1	0	0	0	0	1	1	0
G 107	0	1	0	0	0	2	2	2	2	1	1	0	0	0	0	1	2	0
G 108	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	0	0
G 109	0	1	0	0	0	2	2	2	2	0	1	0	0	0	0	1	1	0
G 110	0	1	0	0	0	2	2	2	2	1	1	0	0	0	1	1	2	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
G 61	0	0	0	0	1	2	2	1	0	0	0	1	1	1	1	2	2	0
G 62	0	0	0	0	1	2	2	0	1	1	0	1	2	0	1	1	1	0
G 63	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	1	1	0
G 64	0	0	0	0	1	2	2	0	0	0	1	1	1	1	1	1	1	0
G 65	0	0	0	0	1	0	2	1	1	1	0	1	1	0	1	1	1	0
G 66	0	0	0	0	1	0	1	1	0	0	0	1	1	0	1	1	1	0
G 67	0	0	0	0	1	2	2	1	1	0	1	1	1	1	1	1	2	0
G 68	0	0	0	0	1	2	2	0	0	0	0	1	2	0	2	2	2	0
G 69	0	0	0	0	1	1	2	1	1	1	1	1	1	1	1	1	1	0
G 70	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0
G 71	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0
G 72	0	0	0	0	1	2	2	0	0	0	0	1	2	0	1	1	1	0
G 73	0	0	0	0	1	2	2	0	0	0	0	1	1	1	2	2	2	1
G 74	0	0	0	0	1	2	2	0	0	0	0	1	1	1	2	2	2	0
G 75	0	0	0	0	1	1	1	1	1	1	0	1	1	0	2	2	2	1
G 76	0	0	0	0	1	0	2	1	1	1	1	1	1	1	1	1	1	0
G 77	0	0	0	0	1	0	2	1	0	1	0	1	1	1	1	1	2	0
G 78	0	0	0	0	1	1	2	1	1	0	1	1	2	0	1	1	2	0
G 79	0	0	0	0	1	1	2	1	0	0	0	0	2	0	1	1	2	0
G 80	0	0	0	0	1	2	2	1	0	1	0	0	2	0	1	2	2	0
G 81	0	0	0	0	1	2	2	1	0	0	0	1	1	0	1	2	2	0
G 82	0	0	0	0	1	1	1	0	0	0	0	1	0	1	1	1	1	1
G 83	0	0	0	0	1	1	1	0	0	0	0	1	0	1	1	1	1	1
G 84	0	0	0	0	1	1	1	0	0	0	0	1	0	1	1	1	1	1
G 85	0	0	0	0	1	1	1	0	0	0	0	1	0	1	1	1	1	1
G 86	0	0	0	0	1	1	1	1	1	1	0	1	2	0	1	2	2	2
G 87	0	0	0	0	1	1	1	1	1	1	0	1	2	0	1	2	2	2
G 88	0	0	0	0	1	1	1	1	1	0	0	1	2	0	1	2	2	2
G 89	0	0	1	0	1	0	0	1	1	1	0	1	1	1	1	2	2	2
G 90	0	0	1	0	1	1	1	1	0	1	0	1	2	0	1	2	2	2
G 91	0	0	0	0	1	0	1	1	1	1	0	1	2	0	2	2	2	2
G 92	0	0	1	0	1	0	1	1	1	1	0	1	1	1	1	2	2	2
G 93	0	0	1	0	1	1	1	1	1	1	0	1	2	0	2	2	2	2
G 94	0	0	1	0	1	1	1	1	1	1	0	1	2	0	1	2	2	2
G 95	0	0	1	0	1	0	1	1	1	1	0	1	2	0	1	2	2	2
G 96	0	0	1	0	1	0	1	1	1	1	1	1	2	0	1	2	2	2
G 97	0	0	1	0	1	0	1	1	1	1	0	1	1	0	1	2	2	2
G 98	0	0	1	0	1	0	1	1	1	1	0	1	1	1	1	2	2	2
G 99	0	0	1	0	1	0	0	1	0	1	0	1	1	0	1	2	2	2
G 100	0	0	1	0	1	0	1	1	0	0	0	1	1	0	1	2	2	2
G 101	0	0	1	0	1	1	1	0	0	0	0	1	1	1	1	2	2	2
G 102	0	0	1	0	1	0	1	1	1	1	0	1	1	1	1	2	2	2
G 103	0	0	1	0	1	0	0	1	1	1	1	1	1	1	1	2	2	2
G 104	0	0	1	0	1	1	1	1	0	1	1	1	2	0	1	2	2	2
G 105	0	0	1	0	1	1	1	1	1	1	0	1	2	0	1	2	2	2
G 106	0	0	1	0	1	0	1	1	1	1	1	1	2	0	1	2	2	2
G 107	0	0	1	0	1	1	2	1	0	1	1	1	2	0	2	2	2	2
G 108	0	0	1	0	1	0	1	1	0	1	0	1	1	1	1	2	2	2
G 109	0	0	1	0	1	1	2	1	0	1	0	1	1	1	2	2	2	2
G 110	0	0	1	0	1	1	1	1	1	1	0	1	1	0	1	2	2	1

TABLE I. (continued).

Strain No.	Character Nos.																	
	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
G 61	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	1	0	0
G 62	0	1	0	1	0	1	1	0	1	0	1	0	0	1	1	1	2	2
G 63	0	1	0	0	0	1	0	0	1	0	0	1	0	1	2	1	1	1
G 64	0	0	0	0	0	0	0	0	0	1	0	0	0	1	2	2	2	2
G 65	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	1
G 66	0	1	0	1	0	0	1	0	1	0	0	0	0	1	1	1	1	1
G 67	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	1
G 68	0	1	0	0	0	0	0	0	0	1	1	0	0	0	2	2	2	2
G 69	0	1	0	0	0	1	0	0	1	1	0	1	0	0	2	2	1	2
G 70	0	0	0	0	0	1	0	0	1	1	0	1	0	1	2	1	1	1
G 71	0	0	0	0	0	1	0	0	1	1	1	1	0	0	1	1	1	1
G 72	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1
G 73	1	2	0	0	0	0	0	0	0	1	1	0	0	0	2	2	1	1
G 74	1	1	0	0	0	0	0	0	0	1	1	0	0	1	2	2	2	2
G 75	1	2	0	0	0	0	0	0	1	1	1	0	0	1	2	1	1	1
G 76	0	0	0	0	0	1	0	0	0	1	1	1	0	0	1	1	1	1
G 77	0	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0	1	1
G 78	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1	1	1	1
G 79	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	1	1	1
G 80	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	2	2	2
G 81	1	1	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1
G 82	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	1	1	1
G 83	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	1	1	1
G 84	1	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1
G 85	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	1	1	1
G 86	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 87	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 88	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 89	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 90	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 91	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 92	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 93	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 94	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 95	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 96	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 97	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 98	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 99	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 100	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 101	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 102	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 103	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 104	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 105	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 106	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 107	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 108	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 109	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1
G 110	2	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	1

TABLE I. (continued).

Strain No.	Character Nos.																	
	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
G 61	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	0
G 62	1	2	1	0	1	2	0	0	1	0	0	0	1	0	0	1	0	0
G 63	1	1	1	1	1	1	0	0	1	0	0	0	1	0	0	1	0	0
G 64	1	2	2	0	1	2	0	0	1	0	0	0	1	0	0	1	0	0
G 65	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	0
G 66	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	2
G 67	1	1	1	0	1	1	0	1	1	0	0	0	1	0	0	1	0	0
G 68	2	2	2	0	1	2	0	1	1	0	0	0	1	0	0	1	0	0
G 69	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0
G 70	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0
G 71	1	1	1	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0
G 72	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0
G 73	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0
G 74	2	2	2	0	1	2	0	0	0	0	0	0	1	0	0	1	0	0
G 75	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	1
G 76	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0
G 77	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	1
G 78	1	1	1	0	1	1	0	0	0	0	0	0	1	0	0	1	1	1
G 79	1	1	1	0	1	1	0	0	0	0	0	0	1	0	0	1	1	1
G 80	1	2	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0
G 81	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	1
G 82	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0
G 83	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0
G 84	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0
G 85	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0
G 86	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 87	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 88	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	1	2
G 89	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 90	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	2
G 91	1	1	1	0	1	1	1	0	0	0	0	0	1	0	0	1	1	2
G 92	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 93	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 94	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 95	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 96	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 97	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 98	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 99	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	1	2
G 100	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	1	2
G 101	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 102	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 103	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	1	2
G 104	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 105	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 106	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 107	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 108	1	1	1	0	1	1	1	0	1	0	0	0	0	0	0	1	1	2
G 109	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2
G 110	1	1	1	0	1	1	1	0	0	0	0	0	1	0	0	1	2	2

TABLE I. (continued).

Strain No.	Character Nos.																		
	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
G 61	1	2	1	0	2	1	0	0	0	2	0	2	2	0	2	0	2	2
G 62	0	2	1	2	0	0	2	0	0	2	0	2	0	0	0	2	0	2
G 63	0	2	2	2	0	0	2	2	0	2	0	2	2	0	2	2	0	0
G 64	0	2	0	2	0	0	0	0	0	0	0	0	0	0	2	0	0	0
G 65	1	2	1	1	2	0	0	2	2	2	0	2	2	1	2	0	2	2
G 66	1	2	2	1	2	0	0	2	2	2	2	2	2	2	2	0	2	2
G 67	0	0	0	0	0	0	2	0	0	0	0	0	2	0	2	0	2	0
G 68	0	0	0	0	0	0	0	0	0	1	0	1	2	0	2	0	0	0
G 69	0	1	0	0	0	0	0	0	0	2	0	2	2	0	2	0	0	0
G 70	0	2	0	2	0	0	0	0	0	2	0	2	2	0	2	0	0	0
G 71	0	2	0	2	0	0	0	0	0	2	0	2	2	0	2	0	0	0
G 72	0	2	2	2	0	0	0	0	0	2	0	2	0	0	2	0	0	0
G 73	0	2	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0
G 74	0	2	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0
G 75	1	2	1	1	2	0	0	0	0	2	0	2	2	0	0	0	2	1
G 76	0	2	0	2	0	0	0	0	0	2	0	0	2	0	2	0	0	0
G 77	1	2	1	2	2	1	2	2	2	2	2	2	2	2	2	0	2	2
G 78	1	2	1	2	1	1	0	2	2	2	2	2	2	2	2	0	2	1
G 79	1	2	1	2	2	1	0	2	2	2	0	2	2	2	2	0	2	1
G 80	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0
G 81	0	1	1	1	2	1	0	0	2	2	0	0	2	0	2	0	2	1
G 82	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	2	0	0
G 83	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	2	0	0
G 84	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	0	0	0
G 85	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	0	0	0
G 86	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 87	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 88	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 89	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 90	0	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 91	1	2	2	2	2	1	0	0	2	2	0	2	2	2	2	2	2	2
G 92	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 93	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 94	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 95	1	2	2	2	2	1	0	0	2	2	0	2	2	2	2	2	2	2
G 96	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 97	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 98	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 99	1	2	2	2	2	0	0	0	2	2	0	2	2	2	2	2	2	2
G 100	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 101	1	2	2	2	1	1	0	2	2	2	0	2	2	2	2	2	2	2
G 102	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 103	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 104	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 105	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 106	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 107	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	0	2	2
G 108	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 109	1	2	2	2	2	1	0	2	2	2	0	2	2	2	2	2	2	2
G 110	2	2	2	2	2	1	0	2	2	2	0	1	2	2	2	2	2	2

TABLE I. (continued).

Strain No.	Character Nos.																
	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
G 61	2	1	1	0	1	0	0	1	1	0	1	0	1	0	0	2	0
G 62	0	2	1	0	0	0	0	0	0	0	1	0	0	0	0	2	0
G 63	2	2	1	1	1	0	0	1	0	0	0	0	2	0	0	2	0
G 64	0	2	1	1	1	0	0	0	0	1	0	0	0	0	0	2	0
G 65	2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 66	2	0	1	1	1	0	0	0	0	0	0	2	0	0	2	2	0
G 67	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 68	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 69	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	2	0
G 70	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 71	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 72	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 73	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 74	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 75	1	2	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 76	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 77	2	2	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 78	1	2	1	1	1	0	0	0	0	0	0	0	2	0	0	2	0
G 79	1	2	1	0	1	0	0	0	1	0	0	0	2	0	0	2	0
G 80	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	2	0
G 81	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 82	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0
G 83	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0
G 84	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0
G 85	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0
G 86	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 87	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 88	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 89	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 90	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 91	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 92	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 93	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 94	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 95	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 96	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 97	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 98	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 99	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 100	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 101	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 102	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 103	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 104	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 105	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 106	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 107	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 108	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 109	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 110	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
G 111	0	0	1	0	0	0	1	0	0	0	0	1	0	1	1	1	1	0
G 112	1	0	0	0	1	0	1	0	0	0	1	1	0	1	1	0	0	0
G 113	1	0	0	0	1	0	1	0	0	0	0	1	0	1	1	1	0	0
G 114	0	0	1	0	0	0	1	0	0	0	0	1	0	1	1	1	0	0
G 115	0	0	0	0	1	0	1	0	0	0	0	1	0	1	1	1	0	0
G 116	0	0	1	0	0	0	1	0	0	0	0	1	0	1	1	1	1	0
G 117	1	0	1	0	0	0	1	0	0	0	0	1	0	1	1	1	0	0
G 118	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0
G 119	1	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0
G 120	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 121	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1
G 122	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1
G 123	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 124	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 125	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 126	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 127	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1
G 128	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1
G 129	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 130	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 131	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
G 132	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
G 1330	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1
G 133T	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1
G 134	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1
G 135	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1
G 136	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1
G 137	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1
G 138	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 139	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
G 140	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1
G 141	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1
G 142	1	1	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0
G 143	1	1	1	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0
G 144	1	1	1	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0
G 145	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0
G 146	0	0	1	0	0	0	1	0	0	0	0	1	0	1	1	1	0	1
G 147	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0
G 148	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0
G 149	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	0	0	0
G 150	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0
G 151	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1
G 152	0	0	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1
G 153	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0
G 154	0	0	1	0	0	0	1	0	0	0	1	0	0	1	1	1	0	1
G 155	0	0	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0
G 156	0	0	1	0	0	1	0	0	0	0	1	1	0	1	1	1	0	1
G 157	0	0	1	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0
G 158	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0
G 159	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
G 111	1	1	0	0	0	2	0	1	1	0	0	0	0	0	1	0	0	1
G 112	1	1	1	0	0	2	0	1	1	0	0	0	1	1	1	0	0	0
G 113	1	1	1	0	0	2	0	1	0	1	0	0	0	1	1	0	0	1
G 114	0	1	0	0	0	2	0	1	1	0	0	0	0	0	1	0	0	1
G 115	0	1	0	0	0	2	0	1	1	0	0	0	1	0	0	0	0	1
G 116	0	1	0	0	0	2	0	1	1	0	0	0	1	0	1	0	0	1
G 117	0	1	0	0	0	2	0	1	1	0	0	0	0	0	0	0	0	1
G 118	1	1	0	0	0	2	0	1	1	0	0	0	1	0	0	0	0	1
G 119	0	1	0	0	0	2	0	1	1	0	0	0	1	0	1	0	0	1
G 120	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0
G 121	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0
G 122	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0
G 123	0	1	1	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0
G 124	0	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0
G 125	0	1	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
G 126	0	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0
G 127	0	1	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
G 128	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0
G 129	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0
G 130	0	1	1	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0
G 131	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0
G 132	0	1	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0
G 1330	0	1	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
G 133T	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0
G 134	0	1	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
G 135	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0
G 136	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0
G 137	0	1	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
G 138	0	1	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
G 139	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
G 140	0	1	1	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0
G 141	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0
G 142	1	1	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0
G 143	1	1	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0
G 144	1	1	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0
G 145	0	1	1	0	0	0	1	0	1	0	0	0	1	1	1	0	1	0
G 146	0	1	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0
G 147	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0
G 148	0	1	1	0	0	0	1	0	1	0	0	0	1	0	1	1	1	1
G 149	1	1	0	0	0	2	0	0	1	0	0	0	1	1	0	0	0	0
G 150	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0
G 151	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0
G 152	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
G 153	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0
G 154	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
G 155	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	1
G 156	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0
G 157	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
G 158	0	1	0	0	0	0	1	0	1	0	0	0	1	1	1	1	0	0
G 159	0	1	1	0	0	1	1	0	0	1	0	0	0	1	0	0	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
G 111	0	0	1	0	0	2	2	2	2	2	1	0	0	0	1	0	0	1
G 112	1	0	1	0	0	2	2	2	2	2	1	0	0	0	1	0	0	1
G 113	0	0	1	0	0	2	2	2	2	2	1	0	0	0	1	0	0	1
G 114	0	0	1	0	0	2	2	2	2	2	2	0	0	0	1	0	0	1
G 115	0	0	1	0	0	2	2	2	2	2	2	0	0	0	1	0	0	1
G 116	0	0	1	0	0	2	2	2	2	2	2	0	0	0	1	0	0	1
G 117	1	1	1	0	0	1	2	2	2	2	1	0	0	0	1	0	0	1
G 118	0	0	1	0	0	0	2	2	2	2	2	0	0	0	1	0	0	1
G 119	0	1	1	0	0	1	2	2	2	2	1	0	0	0	1	0	0	1
G 120	0	1	0	0	0	1	1	2	2	2	1	0	0	0	1	1	0	0
G 121	0	0	0	0	0	0	1	2	2	2	1	0	0	0	1	1	0	0
G 122	1	1	0	0	0	0	1	2	2	2	1	0	0	0	0	1	0	0
G 123	0	0	0	0	0	1	1	2	2	2	2	0	0	0	0	1	0	0
G 124	0	1	0	1	0	2	2	2	2	2	2	0	0	0	1	1	0	0
G 125	0	1	0	1	0	2	2	2	2	2	0	0	0	0	1	1	1	0
G 126	0	1	0	1	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 127	0	1	0	1	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 128	0	1	0	1	0	1	2	2	2	2	2	0	0	0	1	1	1	0
G 129	0	1	0	0	0	2	1	2	2	2	1	0	0	0	1	1	0	0
G 130	0	1	0	0	0	0	1	2	2	2	1	0	0	0	0	1	1	0
G 131	0	1	0	0	0	0	1	2	2	2	1	0	0	0	1	1	0	0
G 132	0	1	0	0	0	0	1	2	2	2	2	0	0	0	1	1	0	0
G 1330	0	1	0	0	0	1	1	2	2	2	1	0	0	0	0	1	0	0
G 133T	0	1	0	0	0	1	1	2	2	2	1	0	0	0	1	1	0	0
G 134	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	0	0
G 135	0	1	0	0	0	0	1	2	2	2	1	0	0	0	0	1	0	0
G 136	0	1	0	0	0	1	2	2	2	2	1	2	0	0	1	1	0	0
G 137	0	0	0	0	0	2	1	2	2	2	1	0	0	0	0	1	0	0
G 138	0	1	0	0	0	2	2	2	2	2	2	2	0	0	1	1	1	0
G 139	0	1	0	0	0	0	2	2	2	2	1	0	0	0	0	1	1	0
G 140	0	0	0	0	0	2	2	2	2	2	2	0	0	0	1	1	1	0
G 141	0	1	0	0	0	1	1	2	2	2	2	0	0	0	0	1	0	0
G 142	0	1	0	0	0	2	2	2	2	2	0	0	0	0	1	1	0	0
G 143	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	0	0
G 144	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	0	0
G 145	1	1	0	0	0	1	2	2	2	2	1	1	0	0	1	1	0	2
G 146	1	1	0	1	0	1	2	2	2	2	1	2	0	0	0	0	0	1
G 147	0	0	0	0	0	0	2	2	2	2	1	0	0	0	0	0	0	0
G 148	1	1	1	0	0	1	2	2	2	2	1	1	0	0	1	1	0	2
G 149	0	0	0	1	0	0	2	2	2	2	1	1	1	1	1	0	0	0
G 150	0	0	0	0	0	1	2	2	2	2	1	0	0	0	0	1	0	0
G 151	0	1	0	0	0	2	2	2	2	2	2	1	0	0	1	1	0	0
G 152	1	1	1	0	0	1	2	2	2	2	2	0	0	0	1	1	0	0
G 153	0	0	0	0	0	2	1	2	2	2	2	0	0	0	1	1	1	0
G 154	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	0	0
G 155	1	1	1	0	0	1	1	2	2	1	1	0	0	0	0	0	0	2
G 156	1	1	0	0	0	1	2	2	2	2	2	0	0	0	1	1	2	0
G 157	0	1	0	0	0	0	2	2	2	2	1	0	0	0	0	0	0	0
G 158	1	1	0	0	0	1	2	2	2	2	1	0	0	0	1	0	0	0
G 159	1	1	0	0	0	0	2	2	2	2	1	0	0	0	0	1	1	1

TABLE I. (continued).

Strain No.	Character Nos.																	
	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
G 111	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	2	2
G 112	1	1	1	0	0	0	0	1	1	0	0	1	1	1	1	1	2	2
G 113	2	2	1	0	0	1	1	1	1	0	0	1	2	0	1	1	2	2
G 114	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	2
G 115	1	1	1	0	0	0	1	1	1	1	0	1	1	1	1	1	1	2
G 116	1	1	1	0	0	0	1	1	1	1	0	1	1	1	1	1	1	2
G 117	1	1	1	0	0	0	1	1	1	1	0	1	1	1	1	1	1	2
G 118	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	2
G 119	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	2
G 120	0	0	0	0	1	1	1	1	1	1	0	1	2	0	2	2	2	0
G 121	0	0	0	0	1	0	1	1	1	0	0	1	0	1	1	2	2	0
G 122	0	0	0	0	1	1	1	1	1	1	0	1	0	1	2	2	2	0
G 123	0	0	0	0	1	1	1	1	1	0	0	1	0	1	1	1	1	0
G 124	0	0	0	0	1	0	1	1	1	1	0	1	1	1	1	2	2	0
G 125	0	0	0	0	1	0	0	1	1	1	0	1	0	1	1	1	1	0
G 126	0	0	0	0	1	0	0	1	1	1	0	1	0	1	1	1	2	0
G 127	0	0	1	0	1	0	1	1	1	1	0	1	0	1	1	1	1	0
G 128	0	0	1	0	1	0	1	1	1	1	0	1	0	1	1	1	1	0
G 129	0	0	0	0	1	1	2	1	1	1	0	1	1	0	1	2	2	0
G 130	0	0	0	0	1	1	2	1	1	1	0	1	1	1	2	2	2	0
G 131	0	0	0	0	1	2	2	1	1	1	0	1	1	1	2	2	2	0
G 132	0	0	1	0	1	1	1	1	1	1	0	1	1	1	1	2	2	0
G 133O	0	0	0	0	1	1	2	1	1	1	0	1	1	1	2	1	2	0
G 133T	0	0	0	0	1	0	2	1	1	0	0	1	1	1	2	2	2	0
G 134	0	0	0	0	1	0	1	1	1	1	0	1	0	1	1	1	2	0
G 135	0	0	0	0	1	1	1	0	0	0	0	1	2	0	2	2	2	1
G 136	0	0	0	0	1	1	1	1	0	0	0	1	1	1	2	2	2	0
G 137	0	0	1	0	1	2	2	0	0	1	0	1	0	1	2	2	2	1
G 138	0	0	0	0	1	0	0	1	1	1	0	1	0	1	1	1	2	0
G 139	0	0	0	0	1	1	1	0	0	0	0	1	2	0	2	2	2	2
G 140	0	0	0	0	1	1	1	1	1	0	0	1	2	0	2	2	2	0
G 141	0	0	0	0	1	2	2	0	0	0	0	1	2	0	2	2	2	0
G 142	0	0	1	0	1	1	1	1	1	0	0	0	1	0	1	1	1	1
G 143	0	0	1	0	1	1	1	1	1	1	0	0	1	0	1	1	1	1
G 144	0	0	1	0	1	2	2	1	0	0	0	0	1	0	1	1	1	1
G 145	2	2	1	0	1	1	1	1	0	0	2	0	2	0	2	2	2	1
G 146	2	2	1	0	0	2	2	0	0	1	0	1	2	0	1	1	1	1
G 147	0	0	1	0	1	1	1	1	1	1	0	1	2	0	2	2	2	2
G 148	2	2	1	0	1	1	1	1	1	1	0	1	2	0	1	1	1	1
G 149	0	0	1	1	0	1	2	1	1	1	0	1	0	1	0	0	0	1
G 150	0	0	0	0	1	1	1	1	1	1	0	1	2	0	2	2	2	2
G 151	0	0	1	0	1	1	2	1	1	1	0	1	1	1	0	1	1	2
G 152	0	0	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	2
G 153	0	0	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1
G 154	0	0	0	0	1	0	1	0	0	1	0	1	2	0	2	2	2	2
G 155	2	2	1	0	0	2	2	0	0	1	0	1	1	1	1	2	2	1
G 156	0	0	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	0
G 157	0	0	1	1	1	0	0	0	0	0	0	1	1	1	2	2	2	2
G 158	0	0	1	0	1	0	2	1	1	1	0	1	1	1	2	2	2	1
G 159	1	2	1	0	1	0	1	1	1	1	2	0	2	0	1	2	2	2

TABLE I. (continued).

Strain No.	Character Nos.																	
	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
G 111	2	2	1	0	1	0	0	0	1	0	0	0	1	0	2	1	1	1
G 112	2	2	1	0	1	0	0	0	1	0	0	0	1	0	1	1	1	1
G 113	2	2	1	1	1	0	0	0	1	0	1	0	1	0	1	1	1	1
G 114	2	2	1	0	1	0	0	0	1	0	0	0	1	0	1	1	1	1
G 115	2	2	1	1	1	0	0	0	1	0	0	0	1	0	2	2	1	1
G 116	2	2	1	1	1	0	0	0	1	1	0	0	1	0	1	1	1	1
G 117	2	2	1	1	1	0	0	0	1	1	1	0	1	0	1	1	1	1
G 118	2	2	1	1	1	0	0	0	1	1	0	0	1	0	2	2	2	2
G 119	2	2	1	1	1	0	0	0	1	1	0	0	1	0	2	1	1	2
G 120	0	2	0	0	0	1	1	0	1	1	1	0	0	1	1	1	1	1
G 121	0	1	0	1	0	1	0	0	1	1	1	0	0	1	2	2	2	2
G 122	0	2	0	0	0	1	1	0	1	1	1	0	0	1	1	1	1	1
G 123	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
G 124	0	0	0	0	0	1	1	0	1	1	1	0	0	1	1	1	1	1
G 125	0	0	0	1	0	1	1	0	1	1	1	0	0	0	1	1	1	1
G 126	0	0	0	1	0	1	1	0	1	1	1	0	0	0	0	1	1	1
G 127	0	0	0	1	0	1	1	0	1	1	1	0	0	1	1	1	1	1
G 128	0	0	0	1	0	1	1	0	1	1	1	0	0	1	1	1	1	1
G 129	1	1	0	1	0	1	0	0	1	1	1	0	0	1	2	1	2	2
G 130	1	1	0	0	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 131	1	2	0	0	0	1	1	0	1	1	1	0	0	1	1	1	1	1
G 132	1	2	0	0	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 1330	1	2	0	0	0	1	0	0	1	1	1	0	0	0	1	1	1	1
G 133T	1	1	0	0	0	1	0	0	1	1	1	0	0	0	2	2	2	2
G 134	0	0	0	0	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 135	2	2	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1	1
G 136	1	2	0	0	0	1	1	0	1	1	1	0	0	0	1	1	1	1
G 137	2	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1
G 138	1	0	0	1	0	1	1	0	1	1	0	0	0	0	1	1	1	1
G 139	2	2	0	1	0	1	1	0	0	1	1	0	0	1	1	2	1	2
G 140	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	1	1
G 141	1	2	0	0	0	1	1	0	0	1	0	0	0	0	2	2	2	2
G 142	2	2	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	1
G 143	2	2	0	1	0	0	1	0	1	0	0	0	0	1	0	1	1	1
G 144	2	2	0	1	0	0	1	0	1	1	0	0	0	1	1	1	1	1
G 145	2	2	0	1	1	0	1	0	0	1	0	0	0	0	2	2	2	2
G 146	2	2	0	1	1	0	1	0	1	1	1	0	0	0	1	1	1	1
G 147	2	2	0	1	1	0	1	0	0	1	1	0	1	0	2	2	2	2
G 148	2	2	0	1	1	0	1	0	1	1	0	0	0	0	1	1	1	1
G 149	2	2	1	1	1	0	0	0	1	0	0	0	1	0	0	0	1	1
G 150	2	2	0	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
G 151	2	2	1	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1
G 152	2	2	0	1	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 153	2	2	0	1	0	0	1	0	1	1	1	0	0	1	1	1	1	1
G 154	2	2	0	0	0	0	1	0	1	0	0	1	0	0	1	1	1	1
G 155	1	2	0	0	1	0	0	0	1	0	0	0	1	0	1	1	1	1
G 156	1	2	1	1	0	1	1	0	1	0	0	0	1	0	0	1	1	1
G 157	2	2	0	1	0	1	0	0	1	1	1	0	0	0	2	1	1	1
G 158	1	1	0	1	0	0	1	0	1	1	0	0	0	0	2	1	1	1
G 159	2	2	1	0	1	0	0	0	1	1	1	0	0	0	1	1	1	1

TABLE I. (continued).

Strain No.	Character Nos.																	
	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
G 111	2	1	2	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 112	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
G 113	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 114	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 115	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 116	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 117	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 118	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 119	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 120	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0
G 121	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	0
G 122	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0
G 123	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	1	0
G 124	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0
G 125	1	1	1	0	1	1	1	0	1	0	0	0	0	0	0	1	1	1
G 126	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	1	1
G 127	1	1	1	0	1	1	1	0	1	0	0	0	0	0	0	1	1	2
G 128	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	1	1
G 129	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	1
G 130	1	1	2	0	1	1	1	0	0	0	0	0	1	0	0	1	0	0
G 131	1	1	1	1	1	1	0	0	0	0	0	0	1	0	0	1	1	1
G 132	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	2	0
G 1330	1	1	1	1	1	1	0	0	0	0	0	0	1	0	0	1	0	0
G 133T	2	2	2	1	2	2	0	0	0	0	0	1	1	0	0	1	0	0
G 134	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	2	2
G 135	1	1	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0
G 136	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0
G 137	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0
G 138	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	1	0
G 139	2	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	2	0
G 140	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	1	2	0
G 141	2	2	2	1	2	2	0	0	0	0	0	1	0	0	0	1	2	0
G 142	1	1	1	0	1	1	0	1	1	0	0	0	0	0	0	1	1	0
G 143	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	1	0
G 144	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0
G 145	2	2	2	1	2	2	0	0	0	1	0	1	1	1	0	1	0	0
G 146	1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0	0	0
G 147	2	2	2	1	1	1	1	0	0	1	0	1	1	0	0	1	2	0
G 148	1	1	1	0	1	1	1	1	0	1	0	1	1	1	0	1	0	0
G 149	0	0	1	1	1	0	1	0	1	1	1	0	0	0	0	0	0	0
G 150	1	1	1	1	1	1	1	0	0	1	0	1	1	0	0	1	0	0
G 151	1	1	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	2
G 152	1	0	1	0	1	1	0	0	0	1	0	1	0	0	0	1	0	2
G 153	1	1	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	2
G 154	1	1	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	2
G 155	1	1	1	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0
G 156	1	1	1	0	1	1	1	0	1	1	0	0	0	0	0	1	0	0
G 157	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
G 158	1	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	0	2
G 159	1	1	1	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
G 111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 114	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
G 115	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
G 116	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
G 117	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0
G 118	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0
G 119	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
G 120	0	2	2	1	2	1	2	2	2	2	0	2	2	0	2	0	2	2
G 121	1	2	1	1	2	1	2	2	2	2	2	2	2	0	2	1	2	2
G 122	0	0	0	1	2	0	0	0	0	2	0	2	2	0	2	0	0	2
G 123	1	2	2	1	2	1	2	0	0	2	0	2	2	0	2	2	0	2
G 124	0	2	0	0	2	0	0	0	0	2	0	2	0	0	2	0	0	2
G 125	1	2	2	1	2	1	0	2	2	2	2	2	2	2	2	0	2	2
G 126	1	2	1	1	2	1	2	2	2	2	2	2	2	1	2	0	2	2
G 127	1	2	1	1	2	1	0	2	2	2	2	2	2	2	2	1	2	2
G 128	1	2	1	1	2	1	0	2	2	2	0	2	2	2	2	1	2	2
G 129	1	2	1	1	0	1	0	0	0	2	0	2	2	2	2	1	2	0
G 130	0	0	0	0	2	0	0	0	0	2	0	2	2	0	2	0	2	2
G 131	1	2	2	0	2	1	0	0	0	2	0	2	2	0	2	0	2	2
G 132	0	2	1	0	2	0	0	0	0	2	0	2	2	0	2	0	2	2
G 1330	0	2	0	0	0	0	0	0	0	2	0	2	2	0	2	0	0	0
G 133T	0	2	0	0	0	0	0	0	0	2	0	2	2	0	1	0	0	0
G 134	1	2	2	1	2	2	2	2	0	2	2	2	2	2	2	0	2	2
G 135	0	2	0	0	0	0	0	0	0	2	2	0	2	0	2	2	0	2
G 136	0	2	2	0	2	0	0	0	0	2	0	2	0	0	2	2	2	2
G 137	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
G 138	0	2	1	2	2	1	2	2	2	2	0	2	2	0	2	2	2	2
G 139	0	2	0	0	2	0	0	2	0	2	0	2	2	2	2	1	2	2
G 140	0	2	1	1	2	1	0	0	0	2	0	2	2	0	2	2	2	2
G 141	0	2	0	0	0	0	0	0	0	2	0	2	2	0	2	0	2	0
G 142	1	1	2	1	2	1	0	0	0	2	2	0	2	0	2	1	2	2
G 143	1	1	2	1	2	1	0	0	0	2	2	0	2	0	2	1	2	2
G 144	0	1	2	0	2	1	0	0	0	2	2	0	2	0	2	1	2	2
G 145	0	2	0	2	2	0	2	0	1	2	2	2	2	0	2	2	0	2
G 146	0	0	0	0	1	0	0	2	0	2	0	0	2	0	0	0	0	2
G 147	0	2	0	0	2	0	0	0	1	2	0	2	0	0	2	2	0	2
G 148	0	0	0	0	2	0	2	2	0	2	0	2	0	0	2	2	0	2
G 149	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
G 150	0	2	0	0	2	0	2	0	2	2	0	2	2	0	0	2	2	2
G 151	0	2	0	0	2	2	0	2	2	1	0	2	2	0	0	0	0	2
G 152	0	2	0	0	2	0	0	0	0	2	0	2	2	2	2	0	0	2
G 153	0	2	0	0	2	2	0	2	2	2	0	2	2	0	2	2	0	2
G 154	0	2	0	0	2	0	2	2	2	2	2	2	2	2	2	2	2	2
G 155	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
G 156	0	2	0	0	0	0	0	2	2	2	0	2	0	0	0	0	2	0
G 157	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
G 158	0	2	0	0	2	0	2	0	2	0	0	2	2	0	2	2	2	2
G 159	0	0	0	0	0	0	0	0	2	2	0	0	0	0	2	0	0	0

Strain No.	Character Nos.																	
	127	128	129	130	131	132	1330	133T	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159
G 111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 120	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 121	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 122	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 123	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 124	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 125	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 126	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 127	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 128	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 129	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 130	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 131	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 132	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 1330	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 133T	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 134	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 135	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 136	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 137	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 138	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 139	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 140	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 141	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 142	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 143	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 144	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 145	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 146	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 147	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 148	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 149	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 150	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 151	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 152	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 153	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 154	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 155	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 156	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 157	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 158	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 159	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I. (continued).

Strain No.	Character Nos.																
	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
G 111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 112	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	1	0
G 113	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
G 114	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
G 115	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
G 116	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0
G 117	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0
G 118	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
G 119	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
G 120	0	2	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0
G 121	0	2	1	0	1	0	0	0	0	0	0	2	0	0	0	2	0
G 122	0	2	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0
G 123	2	2	1	0	1	0	0	0	0	0	0	1	0	0	0	2	0
G 124	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 125	2	2	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 126	0	2	1	1	1	0	0	0	0	0	0	1	0	0	0	2	0
G 127	2	2	1	1	1	0	0	0	0	0	0	2	0	0	2	2	0
G 128	2	2	1	1	1	0	0	0	0	0	0	2	0	0	2	2	0
G 129	0	2	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0
G 130	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 131	0	2	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0
G 132	0	2	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 133O	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 133T	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0
G 134	2	2	1	1	1	0	0	0	0	0	0	2	0	0	2	2	0
G 135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
G 136	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	0
G 137	0	1	0	0	0	0	0	0	0	0	0	2	0	1	0	2	0
G 138	1	2	1	0	1	0	0	0	0	0	0	1	0	0	0	2	0
G 139	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	2	0
G 140	1	1	1	0	1	0	0	0	0	0	0	0	0	0	2	2	0
G 141	0	2	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0
G 142	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	2	1
G 143	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	2	1
G 144	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	2	1
G 145	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 146	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
G 147	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0
G 148	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0
G 149	0	0	1	1	0	0	0	0	0	1	0	1	0	1	0	2	0
G 150	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0
G 151	0	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 152	0	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	1
G 153	0	0	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 154	2	2	1	1	1	0	0	0	0	0	0	2	0	0	0	2	0
G 155	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
G 156	2	0	1	1	1	0	0	0	0	1	1	1	2	1	0	2	0
G 157	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 158	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0
G 159	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
G 160	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0
G 161	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	0	0	0
G 162	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0
G 163	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0
G 164	0	0	1	0	0	1	0	0	0	0	1	1	0	1	1	1	0	1
G 165	0	0	1	0	0	1	0	0	0	0	1	1	0	1	1	1	0	0
G 166	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	1	0
G 167	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	1	0
G 168	1	0	1	0	0	0	1	0	0	0	1	1	0	1	1	0	0	0
G 169	0	0	1	0	0	1	0	0	0	0	1	1	0	1	1	1	0	0
G 170	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0
G 171	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	1	1	1
G 172	0	0	1	0	0	0	1	0	1	0	1	1	0	1	0	1	0	0
G 173	0	0	1	0	0	0	1	0	1	0	1	1	0	1	0	1	0	1
G 174	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0	1	1	0
G 175	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	1
G 176	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	1
G 177	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1
G 178	0	0	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1
G 179	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	1
G 180	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1
G 181	0	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0	0	1
G 182	0	0	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1
G 183	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 184	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 185	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 186	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0
G 187	0	1	1	0	0	1	0	0	0	0	1	0	0	1	0	1	1	0
G 188	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	1	0
G 189	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	1
G 190	0	1	1	0	0	0	1	0	0	1	1	0	0	1	0	1	1	0
G 191	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0
G 192	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0
G 193	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0
G 194	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0
G 195	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0
G 196	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	1	1
G 197	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	1	1
G 198	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	1	1
G 199	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	1	1
G 200	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1	1
G 201	0	0	0	0	1	0	1	0	0	0	1	1	0	1	1	1	1	1
G 202	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	1	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
G 160	0	1	1	1	0	1	1	0	1	0	0	0	1	1	1	0	0	0
G 161	0	1	1	0	0	1	1	0	0	1	0	0	0	1	0	0	0	0
G 162	0	1	0	0	0	0	1	0	1	0	0	0	1	1	1	1	0	0
G 163	0	1	0	1	0	0	1	0	1	0	0	0	0	1	1	1	0	0
G 164	0	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
G 165	0	1	0	0	0	0	1	0	1	0	0	0	1	1	1	1	0	0
G 166	0	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0	0	1
G 167	0	1	0	0	0	2	0	0	1	0	0	1	1	0	0	0	0	0
G 168	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0
G 169	0	1	0	0	0	1	1	0	1	0	0	0	0	1	1	1	0	0
G 170	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1
G 171	0	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0
G 172	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 173	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
G 174	0	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0
G 175	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
G 176	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0
G 177	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0
G 178	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1
G 179	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
G 180	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1
G 181	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1
G 182	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 183	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
G 184	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
G 185	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
G 186	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
G 187	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
G 188	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
G 189	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
G 190	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
G 191	0	1	1	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0
G 192	0	1	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0
G 193	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 194	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
G 195	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
G 196	0	1	1	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0
G 197	0	1	1	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0
G 198	0	1	1	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0
G 199	0	1	1	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0
G 200	0	1	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	0
G 201	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0
G 202	0	1	0	0	0	2	1	0	1	0	0	0	1	1	1	0	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
G 160	0	1	0	0	1	2	2	2	2	2	1	0	0	0	1	1	0	1
G 161	0	0	0	1	1	1	2	2	2	2	1	0	0	0	1	0	0	0
G 162	1	1	1	0	0	2	2	2	2	1	1	0	0	0	0	0	0	1
G 163	1	1	1	0	0	0	2	2	2	2	1	0	0	0	0	0	0	0
G 164	1	1	1	0	0	0	2	2	2	2	1	0	0	0	1	1	2	0
G 165	0	0	0	0	0	2	2	2	2	1	1	0	0	0	0	0	0	0
G 166	1	1	1	1	0	2	2	2	2	2	1	0	0	0	0	0	1	1
G 167	1	1	0	0	0	1	1	2	2	2	2	2	0	0	1	0	0	1
G 168	0	0	0	0	0	1	2	2	2	1	1	0	0	0	0	0	0	1
G 169	0	1	0	1	0	2	2	2	2	2	1	0	0	0	0	1	0	0
G 170	0	0	0	1	0	2	2	2	2	2	1	1	0	0	0	0	1	1
G 171	1	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	0	1
G 172	0	1	0	0	0	2	1	2	2	2	1	0	0	0	1	1	0	0
G 173	1	1	0	0	0	1	1	2	2	2	1	0	0	0	1	1	0	0
G 174	0	1	0	0	0	1	1	2	2	2	1	0	0	0	1	1	0	0
G 175	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	2	0
G 176	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	2	0
G 177	1	1	0	0	0	1	2	2	2	2	2	0	0	0	1	1	2	0
G 178	1	1	0	0	0	1	2	2	2	2	1	1	0	0	1	1	2	0
G 179	0	0	0	1	0	1	2	2	2	2	1	0	0	0	0	0	0	1
G 180	0	0	0	0	0	2	2	2	2	2	2	0	0	0	0	1	0	0
G 181	1	1	1	0	0	1	2	2	2	2	1	0	0	0	1	0	0	1
G 182	1	0	0	1	1	1	2	2	2	2	1	2	0	0	0	0	1	1
G 183	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	0	0
G 184	0	0	0	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 185	0	0	0	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 186	0	0	0	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 187	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	0	0
G 188	0	0	0	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 189	1	1	0	0	0	1	2	2	2	2	2	0	0	0	1	1	2	0
G 190	0	0	0	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 191	0	1	0	0	0	2	2	2	2	2	1	0	0	0	1	1	1	0
G 192	0	0	0	0	0	1	2	2	2	2	1	0	0	0	0	1	0	0
G 193	0	0	0	0	0	1	2	2	2	2	1	0	0	0	0	1	0	0
G 194	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	0	0
G 195	0	0	0	0	0	2	2	2	2	2	1	0	0	0	1	1	0	0
G 196	0	0	1	0	0	0	2	2	2	2	1	0	0	0	1	1	0	0
G 197	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	1	0
G 198	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	1	0
G 199	0	0	0	0	0	0	2	2	2	2	1	1	0	0	1	1	1	0
G 200	1	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	1	0
G 201	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	0	0
G 202	1	1	0	0	0	1	2	2	2	2	1	0	0	0	1	1	1	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
G 160	1	2	1	0	1	0	1	1	1	1	0	1	2	0	0	0	1	1
G 161	1	1	1	0	1	0	0	1	1	1	0	1	1	1	0	0	1	2
G 162	1	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	1	1
G 163	1	1	1	0	1	0	1	1	1	1	0	1	1	1	0	1	1	2
G 164	1	1	1	1	0	0	0	1	0	0	0	1	2	0	0	1	2	2
G 165	1	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	1
G 166	1	1	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	1
G 167	1	1	1	0	0	1	1	0	0	0	0	1	0	1	2	2	2	2
G 168	1	1	1	0	0	0	1	1	0	1	1	1	1	1	0	1	1	1
G 169	0	0	0	0	1	0	1	1	1	1	0	1	1	1	0	1	1	1
G 170	1	1	1	0	1	0	0	1	1	1	0	1	1	1	0	0	1	1
G 171	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	1
G 172	0	0	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	0
G 173	0	0	0	0	1	2	2	1	1	0	1	1	1	0	1	1	1	0
G 174	0	0	0	0	1	2	2	1	1	0	1	1	1	1	1	1	1	0
G 175	0	0	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	0
G 176	0	0	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	0
G 177	0	0	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	0
G 178	0	0	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	1
G 179	1	2	1	0	1	0	0	1	1	1	1	1	1	0	0	0	1	1
G 180	0	0	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	1
G 181	1	1	1	1	1	0	0	1	1	1	1	1	2	0	1	1	1	1
G 182	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
G 183	0	0	0	0	1	1	2	1	1	1	0	1	0	1	2	2	2	0
G 184	0	0	0	0	1	0	1	1	1	1	0	1	0	1	2	2	2	0
G 185	0	0	0	0	1	1	2	1	1	1	0	1	0	1	2	2	2	0
G 186	0	0	0	0	1	2	2	1	1	1	0	1	0	1	2	2	2	0
G 187	0	0	0	0	1	1	1	1	1	0	0	1	0	1	2	2	2	1
G 188	0	0	0	0	1	2	2	1	1	1	0	1	0	1	2	2	2	1
G 189	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	1	1	0
G 190	0	0	0	0	1	1	1	0	0	0	0	1	0	1	2	2	2	1
G 191	0	0	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1
G 192	0	0	0	0	1	1	1	1	1	1	0	1	0	1	2	2	2	2
G 193	0	0	0	0	1	1	1	1	1	0	0	1	0	1	2	2	2	0
G 194	0	0	0	0	1	1	1	1	1	1	0	1	0	1	2	2	2	0
G 195	0	0	0	0	1	0	0	1	1	1	0	1	0	1	2	2	2	0
G 196	0	0	0	0	1	1	1	1	0	1	0	1	2	0	2	2	2	2
G 197	0	0	0	0	1	1	1	0	0	1	0	1	2	0	2	2	2	2
G 198	0	0	0	0	1	1	2	0	0	1	0	1	2	0	2	2	2	1
G 199	0	0	0	0	1	1	1	0	0	1	0	1	2	0	2	2	2	2
G 200	0	0	0	0	1	1	1	0	0	1	0	1	2	0	2	2	2	2
G 201	0	0	1	0	0	1	1	1	1	1	0	1	2	0	0	1	2	2
G 202	0	0	0	1	1	2	2	0	0	0	0	1	2	0	2	2	2	2

TABLE I. (continued).

Strain No.	Character Nos.																	
	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
G 160	2	2	0	1	1	0	1	0	1	1	1	0	1	0	1	1	1	1
G 161	2	2	1	1	1	0	1	1	1	1	0	0	1	0	1	1	1	1
G 162	2	2	1	1	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 163	2	2	1	1	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 164	2	2	1	1	0	0	1	0	1	1	1	0	0	0	2	2	2	2
G 165	1	2	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
G 166	2	2	1	1	1	0	0	0	1	1	1	0	1	0	1	1	1	1
G 167	2	2	1	1	1	0	0	0	1	1	1	0	1	0	0	1	1	1
G 168	1	1	1	0	0	0	0	0	1	1	1	0	1	0	2	2	2	2
G 169	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
G 170	1	1	1	1	1	0	0	0	1	0	0	0	1	0	1	1	1	1
G 171	1	2	0	1	1	0	0	0	0	0	1	0	1	0	1	1	1	1
G 172	0	1	0	0	0	0	0	0	1	0	0	0	0	1	2	2	2	1
G 173	0	1	0	0	0	0	0	0	1	0	0	0	0	1	2	2	2	1
G 174	1	1	0	0	0	0	0	0	1	0	0	0	0	1	2	2	2	1
G 175	1	2	1	1	0	1	0	0	1	0	0	0	1	0	1	1	1	1
G 176	1	1	1	1	1	0	0	0	1	0	1	0	1	0	1	1	1	1
G 177	1	1	1	1	0	1	0	0	1	1	1	0	1	0	1	1	1	1
G 178	2	2	1	1	0	1	0	0	1	0	1	0	1	0	1	1	1	1
G 179	2	2	1	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1
G 180	1	2	1	1	0	1	0	0	1	0	0	0	1	0	1	1	1	1
G 181	1	1	1	0	1	0	0	0	1	0	0	0	1	1	1	1	1	1
G 182	1	2	1	1	1	0	0	0	1	0	0	0	1	0	2	1	2	2
G 183	2	2	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	1
G 184	1	2	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1
G 185	1	2	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	1
G 186	1	2	0	1	0	1	0	0	1	0	0	0	0	1	1	1	1	1
G 187	1	2	0	0	0	1	0	0	1	0	0	0	0	1	1	1	1	1
G 188	1	2	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1
G 189	0	0	0	0	0	0	1	0	1	1	1	0	0	0	1	1	1	1
G 190	1	2	0	1	0	1	0	0	0	0	0	0	0	1	1	1	1	1
G 191	1	2	0	1	0	0	1	0	1	1	1	0	0	0	2	2	1	2
G 192	1	2	0	1	0	1	0	0	1	0	0	0	0	1	1	1	1	1
G 193	1	2	0	0	0	1	0	0	1	0	0	0	0	1	1	1	1	1
G 194	1	2	0	0	0	1	0	0	1	0	0	0	0	1	1	1	1	1
G 195	1	2	0	1	0	1	0	0	1	0	0	0	0	1	1	1	1	1
G 196	2	2	0	1	0	1	1	0	0	0	0	0	0	0	2	2	2	2
G 197	2	2	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1	1
G 198	2	2	0	0	0	1	1	0	0	0	0	0	0	0	2	2	2	2
G 199	2	2	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1	1
G 200	2	2	0	0	0	1	1	0	0	0	0	0	0	0	2	2	2	2
G 201	2	2	0	0	0	1	1	0	0	1	1	0	0	1	1	1	1	1
G 202	2	2	0	0	1	0	1	0	1	0	0	0	0	1	2	1	2	2

TABLE I. (continued).

Strain No.	Character Nos.																	
	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
G 160	1	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	2	0
G 161	1	1	1	0	1	1	0	0	1	0	0	1	1	0	1	0	0	0
G 162	1	1	1	1	1	1	0	0	1	0	0	1	0	0	0	0	0	2
G 163	1	1	1	0	1	1	0	0	1	0	0	1	0	0	0	1	0	2
G 164	1	1	2	1	1	1	1	0	0	0	0	1	1	0	0	1	0	0
G 165	1	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0
G 166	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	1
G 167	1	0	1	1	1	0	1	0	1	0	0	0	0	0	1	0	0	0
G 168	2	2	2	1	2	2	1	0	0	0	0	1	0	0	0	0	0	0
G 169	1	1	1	0	1	1	1	0	1	0	0	1	0	0	0	1	0	0
G 170	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0
G 171	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	2
G 172	1	2	1	0	2	2	0	0	0	0	0	0	0	0	1	0	0	0
G 173	1	2	1	0	2	2	0	0	0	0	0	0	0	0	0	1	0	0
G 174	1	2	1	0	2	2	0	0	0	0	0	0	0	0	0	1	0	0
G 175	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0	0
G 176	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	1	0	0
G 177	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0
G 178	1	1	1	0	1	1	1	0	1	1	0	0	0	0	0	1	0	0
G 179	1	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0
G 180	1	1	1	1	1	1	1	0	0	1	0	0	1	0	0	1	0	0
G 181	1	1	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0
G 182	2	1	2	1	2	2	2	0	1	1	1	0	1	0	0	0	0	0
G 183	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
G 184	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 185	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 186	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 187	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 188	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 189	1	1	1	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0
G 190	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
G 191	2	2	2	0	2	2	0	1	1	0	0	0	0	0	0	1	1	0
G 192	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 193	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 194	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
G 195	1	1	1	0	0	1	2	0	1	0	0	0	0	0	0	1	0	0
G 196	2	2	2	2	1	1	0	0	0	0	0	1	1	0	0	1	2	0
G 197	1	1	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	0
G 198	2	2	2	1	1	1	0	0	1	0	0	1	1	0	0	1	2	0
G 199	1	1	1	0	1	1	0	0	1	0	0	1	1	0	0	1	2	0
G 200	1	2	2	2	2	2	0	0	0	0	0	1	1	0	0	1	2	0
G 201	1	1	1	1	1	1	1	0	0	1	0	1	1	0	0	1	0	0
G 202	1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	1	0	0

TABLE I. (continued).

Strain No.	Character Nos.																	
	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
G 160	0	0	2	0	2	0	2	0	2	2	0	2	2	0	2	2	2	2
G 161	0	0	0	0	2	0	0	0	2	0	2	2	0	0	2	0	0	2
G 162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
G 163	0	0	0	0	1	0	2	0	2	0	1	2	0	0	0	2	0	0
G 164	0	0	2	0	2	0	0	2	2	0	0	0	2	0	0	0	2	1
G 165	0	2	2	0	2	0	2	0	0	0	0	0	2	0	0	0	2	2
G 166	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
G 167	0	1	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0
G 168	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 169	0	2	2	2	2	0	2	2	0	2	2	2	2	0	2	2	2	2
G 170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 171	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	0	2	0
G 172	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	0	2	0
G 173	0	1	0	0	0	0	0	0	0	2	0	0	2	0	2	0	2	0
G 174	0	0	0	0	0	0	0	0	0	2	0	0	2	0	2	0	2	0
G 175	0	0	0	0	0	0	0	0	2	2	0	0	2	0	2	0	2	0
G 176	0	2	0	0	0	1	0	2	2	2	0	2	2	0	2	0	2	0
G 177	0	2	0	0	0	0	0	2	2	2	0	2	2	0	2	0	2	0
G 178	0	2	0	0	0	0	0	2	2	2	0	2	2	0	2	0	2	0
G 179	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 180	0	2	0	0	0	0	0	0	0	2	0	2	2	0	2	0	2	0
G 181	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
G 182	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 183	0	2	0	0	0	0	0	0	0	1	0	2	0	0	2	0	0	2
G 184	0	2	0	0	0	0	0	0	0	1	0	2	0	0	2	0	0	2
G 185	0	2	0	0	0	0	0	0	0	2	0	2	2	0	2	0	0	0
G 186	0	2	0	0	0	0	0	0	0	1	0	2	2	0	2	0	0	1
G 187	0	2	0	0	0	0	0	0	0	1	0	2	0	0	2	0	0	1
G 188	0	2	0	0	0	0	0	0	0	1	0	2	0	0	2	0	0	1
G 189	0	2	0	2	2	0	0	2	0	2	0	2	2	2	2	2	2	0
G 190	0	2	0	0	0	0	0	0	0	2	0	2	2	0	2	2	2	2
G 191	0	2	2	1	2	0	0	0	2	2	2	0	0	0	2	0	2	2
G 192	0	2	0	0	0	0	0	0	0	1	0	2	0	0	2	0	0	2
G 193	0	2	0	0	0	0	0	0	0	2	0	2	0	0	2	0	0	2
G 194	0	2	0	0	0	0	0	0	0	1	0	2	0	0	2	0	0	2
G 195	0	2	0	0	0	0	0	0	0	1	0	2	0	0	2	0	2	2
G 196	0	2	1	1	2	0	0	0	0	2	0	2	2	0	2	2	2	2
G 197	0	2	1	1	1	0	0	0	0	2	0	2	2	0	2	0	2	2
G 198	0	2	1	1	1	0	0	0	0	2	0	2	2	0	2	2	2	2
G 199	0	2	1	1	1	0	0	0	0	2	0	2	2	0	2	2	2	2
G 200	0	2	2	1	1	0	0	0	0	2	0	2	2	0	2	2	2	2
G 201	0	0	2	2	2	2	0	0	0	2	0	0	2	0	2	0	2	2
G 202	0	0	0	2	2	0	0	2	2	2	0	0	2	0	2	0	2	2

TABLE I. (continued).

Strain No.	Character Nos.																
	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
G 160	0	2	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0
G 161	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
G 162	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 163	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
G 164	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
G 165	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 166	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0
G 167	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0
G 168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 169	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 170	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0
G 171	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
G 172	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 173	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 174	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 175	2	0	1	1	1	0	0	1	0	2	0	2	2	1	2	2	0
G 176	2	0	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0
G 177	0	0	1	1	1	0	0	1	0	0	2	1	1	0	0	2	0
G 178	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	2	0
G 179	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 180	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
G 181	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 182	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
G 183	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G 184	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 185	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 186	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	2	0
G 187	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 188	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 189	1	2	1	1	1	0	0	0	0	0	0	0	0	0	2	0	0
G 190	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 191	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 192	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 193	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 194	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 195	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2	0
G 196	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 197	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 198	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0
G 199	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 200	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0
G 201	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G 202	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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