Identification of Bacteria by Computer: Identification of Reference Strains

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(Received 8 November 1972; revised 19 March 1973)

SUMMARY

The results of the identification of 1079 reference strains of Gram-negative, aerobic, rod-shaped bacteria by a probabilistic method in a computer are given. Comparison of identification by conventional methods and by computer showed that 90.8 % of fermentative and 82.1 % of non-fermentative strains could be identified on the best available probability matrix. Many of these strains were atypical and had caused difficulty in identification in the medical diagnostic laboratory. The implications of various factors in successful computer identification are discussed and the results are given for each taxon by genus and species.

INTRODUCTION

Dybowski & Franklin (1968) gave the first detailed account of bacterial identification by a probabilistic method and were uncertain of its value. Lapage, Bascomb, Willcox & Curtis (1970) successfully identified 70 to 80 % of 279 freshly isolated strains examined in both a limited number of tests and in all the tests included in their survey. General aspects of probabilistic identification are discussed in Lapage, Bascomb, Willcox & Curtis (1973) and the mathematical model is described in Willcox, Lapage, Bascomb & Curtis (1973).

In this paper are presented the results obtained in the identification of 1079 reference strains of bacteria by a probabilistic method used in a computer (Lapage *et al.* 1970, 1973).

METHODS

Overall procedure. As a model for trials of computer identification, Gram-negative, aerobic, rod-shaped bacteria which grew on nutrient agar at 37 or 22 °C were chosen. A matrix was constructed which gave the probability of a strain of any given taxon yielding a positive result in each of the chosen tests (Tables 1, 2). Individual strains were then tested and identified on the basis of these results.

Taxa. The taxa chosen for the matrix are given in Tables 1 and 2. Table 1 contains those taxa which gave a fermentative result in the oxidation/fermentation test (O/F test) of Hugh & Leifson (1953), while the bacteria in Table 2 gave either an oxidative result, produced no acid in the test, or gave an alkaline reaction. They are referred to as non-fermentative bacteria.

The taxa were selected chiefly on their medical importance and as those likely to occur

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Table 2. *Matrix of taxa versus tests for non-fermentative bacteria*

Each entry is the estimated percentage probability that a strain of the relevant taxon will give a positive result in the test concerned.

These probabilities (Matrix 68) were, with minor changes, those used during the course of this study. Some of the probabilities differ from the values that would be allotted if the results of tests on strains received after 1968 were included. These should be taken into consideration in a future revision of the matrix. To publish a further revision of the matrix at this stage would be misleading as we have no knowledge of the identification rate which would be achieved with such a matrix. However, some of the values given in Tables 1 and 2 are not those which we would recommend for diagnostic usage under the specified conditions of testing.

	<i>Acinetobacter anitratus</i>	<i>A. Iwoffii</i>	<i>Alcaligenes faecalis</i>	<i>Bordetella bronchiseptica</i>	<i>B. parapertussis</i>	<i>Chromobacterium lividum</i>	<i>Comamonas percolans</i>	<i>Flavobacterium meningosepticum</i>	<i>Moraxella</i> spp.	<i>Pseudomonas aeruginosa</i>	<i>P. fluorescens</i>	<i>P. mallei</i>	<i>P. maltophilia</i>	<i>P. pseudomallei</i>
Motility at 37 °C	1	1	80	99	1	1	99	1	1	99	25	1	95	99
Motility at RT	1	1	80	99	1	99	99	1	1	99	99	1	99	99
Growth at 37 °C	99	99	99	99	99	1	99	99	99	99	75	99	99	99
Growth at RT	99	99	99	99	30	99	99	99	10	99	99	75	99	99
MacConkey agar, growth	99	99	99	99	15	75	99	90	25	99	99	1	99	95
Catalase, presence	99	99	99	99	99	99	99	99	99	99	99	99	99	99
Oxidase, presence	1	1	60	95	5	99	99	99	75	99	99	1	50	99
Gelatin liquefaction within 5 days	20	1	20	1	1	1	90	1	95	50	1	99	99	99
Gelatin liquefaction within 28 days	50	1	60	1	1	99	20	99	50	99	75	80	99	99
Hugh & Leifson test, oxidative	99	1	1	1	1	95	1	99	1	99	99	99	40	99
Hugh & Leifson test, fermentative	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hugh & Leifson test, alkaline	1	50	99	99	99	1	99	1	1	1	1	1	80	1
Simmons' citrate, growth	90	50	99	95	1	90	60	1	1	99	99	15	30	99
KCN medium, growth	50	1	99	99	1	15	1	1	1	75	25	1	50	99
Gluconate reduction	1	1	1	1	1	1	1	1	1	99	50	1	1	1
Malonate utilization	50	1	1	1	1	50	20	1	1	99	15	1	50	1
Deamination of phenylalanine	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Urease, presence	50	1	1	99	99	50	1	1	1	99	75	70	1	50
Indole production	1	1	1	1	1	1	1	25	1	1	1	1	1	1
H ₂ S production (TSI)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H ₂ S production (lead acetate paper)	1	1	40	1	1	1	20	25	1	1	1	15	75	1
Arginine, dihydrolase	1	1	1	5	1	1	20	1	1	99	99	90	1	99
Lysine, decarboxylase	1	1	1	1	1	1	1	1	1	1	1	1	50	1
Ornithine, decarboxylase	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Methyl red at 37 °C	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Methyl red at RT	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Voges-Proskauer at 37 °C	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Voges-Proskauer at RT	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Nitrate reduction	1	1	20	99	1	95	80	1	90	99	25	90	60	99
Selenite reduction	10	1	20	1	1	1	1	1	1	99	1	1	10	1
Gas production from glucose	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Production of acid from:														
Glucose	99	1	1	1	1	15	1	1	1	25	50	25	1	99
Adonitol	1	1	1	1	1	1	1	1	1	1	1	1	1	50
Arabinose	90	1	1	1	1	10	1	1	1	1	1	1	1	50
Cellobiose	1	1	1	1	1	1	1	1	1	1	1	1	1	50
Dulcitol	1	1	1	1	1	1	1	1	1	1	1	1	1	5
Glycerol	1	1	1	1	1	10	1	1	1	1	1	1	1	99
Inositol	1	1	1	1	1	1	1	1	1	1	1	1	1	75
Lactose	1	1	1	1	1	1	1	1	1	1	1	1	1	50
ONPG*	1	1	1	1	1	10	1	99	1	1	1	1	25	1

Table 1. Matrix of taxa versus tests for fermentative bacteria

[illegible]

These probabilities (Matrix 68) were, with minor changes, those used during the course of this study. Some of the probabilities differ from the values that would be allotted if the results of tests on strains received after 1968 were included. These should be taken into consideration in a future revision of the matrix. To publish a further revision of the matrix at this stage would be misleading as we have no knowledge of the identification rate which would be achieved with such a matrix. However, some of the values given in Tables 1 and 2 are not those which we would recommend for diagnostic usage under the specified conditions of testing. Each entry is the estimated percentage probability that a strain of the relevant taxon will give a positive result in the test concerned.

TSI, triple sugar iron medium.

* β -galactosidase

Identification of reference strains

Table 2. (cont.)

	<i>Acinetobacter anitratus</i>	<i>A. Iwoffii</i>	<i>Alcaligenes faecalis</i>	<i>Bordetella bronchiseptica</i>	<i>B. parapertussis</i>	<i>Chromobacterium lividum</i>	<i>Comamonas percolans</i>	<i>Flavobacterium meningosepticum</i>	<i>Moraxella spp.</i>	<i>Pseudomonas aeruginosa</i>	<i>P. fluorescens</i>	<i>P. mallei</i>	<i>P. maltophilia</i>	<i>P. pseudomallei</i>
Maltose	1	1	1	1	1	10	1	1	1	1	1	1	1	99
Mannitol	1	1	1	1	1	1	1	1	1	1	1	1	1	10
Raffinose	1	1	1	1	1	1	1	1	1	1	1	1	1	10
Rhamnose	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Salicin	1	1	1	1	1	1	1	1	1	1	1	1	1	50
Sorbitol	1	1	1	1	1	10	1	1	1	1	1	1	1	5
Starch	1	1	1	1	1	1	1	1	1	1	1	1	1	50
Sucrose	1	1	1	1	1	1	1	1	1	1	1	1	1	50
Trehalose	1	1	1	1	1	1	1	1	1	1	1	1	1	75
Xylose	90	1	1	1	1	10	1	1	1	1	1	1	1	50
Dirty coloured pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	20
Brown pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Violet pigment	1	1	1	1	1	80	1	1	1	1	1	1	1	1
Green pigment	1	1	1	1	1	1	1	1	1	50	1	1	1	1
Yellow pigment	1	1	1	1	1	1	1	50	1	5	25	1	1	1
Red pigment	1	1	1	1	1	1	1	1	1	5	1	1	1	1

* β -galactosidase. RT, room temperature or incubator at 22 or 30 °C. TSI, triple sugar iron medium.

in medical specimens. The majority are recognized species, genera or subgenera. One had been informally named the 'R.M. bacterium' (Ross, Rucker & Ewing, 1966) and others were taxa specially constructed for use in the matrix but not recognized in classical taxonomy by that name, e.g. *Citrobacter matrix* biotype 2, *Klebsiella* unnamed group and *Pasteurella multocida* matrix biotype 2.

Tests. Since identification was to be made both on the results from our own and other laboratories (Lapage *et al.* 1973), the 48 tests chosen (Table 1) were those considered easy to read, reproducible, least affected by subjective interpretation and commonly used in the medical diagnostic bacteriological laboratory. In practice, 56 test results were available, 48 tests were set up of which one, pigmentation, if present, provided a choice of six possible colours, gelatin liquefaction provided two results and the O/F test three results. These 56 test results were allotted probabilities for each taxon in the matrix (Tables 1, 2).

Methods. The media and methods used correspond in general to those described by Cowan & Steel (1965) and are given in Bascomb, Lapage, Willcox & Curtis (1971). All tests were read at 1, 2 and finally at 5 days, except for gelatin liquefaction which was read up to 28 days.

Coding of tests. Most of the tests were recorded as four-state (+, $\pm$, $\mp$, -). Pigment was coded as absent or present, when it was then coded from a choice of six colours (Table 1). Acid production from carbohydrates was allowed three states (acid, weak acid or no acid) and gas production from glucose three states (gas, little gas or no gas). The O/F test was coded as three-state; oxidative, fermentative or alkaline. All coded characters were stored in the computer, and before the calculations of the identification scores, ' $\pm$ ', 'weak acid' and 'a little gas' were equated with '+'; and ' $\mp$ ' was equated with '-' so that the characters

Table 3. *Identification of fermentative bacteria*

Taxon	No. of strains (1)	Correctly identified by computer (2)		No. of bio-patterns (3)	No. of constant tests in matrix* (4)	Separation from nearest taxon† (5)	Distribution of strains by the number of their test results which differ from the matrix‡ (6)					
		No.	%				0	1	2	3	≥ 4	
<i>Actinobacillus equuli</i>	7	6	86	5	43	0	6	—	1 (1)	—	—	
<i>A. lignieresii</i>	13	10	77	9	34	0	10 (1)	3 (2)	—	—	—	
<i>Aeromonas formicans</i>	11	10	91	10	39	0	8	2 (1)	1	—	—	
<i>A. hydrophila</i>	16	15	94	15	31	0	16 (1)	—	—	—	—	
<i>A. salmonicida</i>	12	12	100	6	43	3	12	—	—	—	—	
<i>Chromobacterium violaceum</i>	14	12	86	13	38	1	9	—	2	1 (1)	2 (1)	
Citrobacter matrix biotype 1	61	57§	93	43	36	0	59 (4)	2	—	—	—	
Citrobacter matrix biotype 2	20	19	95	20	30	0	17 (1)	3	—	—	—	
<i>Edwardsiella tarda</i>	9	9	100	5	47	3	2	7	—	—	—	
<i>Enterobacter aerogenes</i>	3	3	100	3	40	1	3	—	—	—	—	
<i>E. cloacae</i>	34	34	100	30	29	1	29	3	2	—	—	
<i>E. liquefaciens</i>	5	5	100	5	34	0	3	2	—	—	—	
<i>Erwinia herbicola</i>	32	26	81	32	32	0	14	5 (1)	3 (2)	7 (2)	3 (1)	
<i>Escherichia adecarboxylata</i>	7	7	100	4	46	2	6	1	—	—	—	
<i>E. coli</i>	60	58§	97	47	32	0	47	10	3 (2)	—	—	
<i>E.:</i> Alkalescens-Dispar group	11	6§	55	11	37	0	6 (2)	5 (3)	—	—	—	
<i>Hafnia alvei</i>	18	17	94	18	33	1	15	3 (1)	—	—	—	
<i>Klebsiella aerogenes</i> and <i>K. oxytoca</i>	89	84§	94	62	31	0	85 (2)	3 (3)	1	—	—	
<i>K. ozaenae</i>	6	6	100	6	34	0	6	—	—	—	—	
<i>K. pneumoniae</i>	5	5	100	2	45	0	4	1	—	—	—	
<i>K. rhinoscleromatis</i>	6	6	100	5	41	0	6	—	—	—	—	
<i>Klebsiella</i> unnamed group	4	4	100	4	40	1	4	—	—	—	—	
<i>Kluyvera</i> spp.	3	3	100	3	40	0	3	—	—	—	—	
<i>Pasteurella haemolytica</i> biotype A	23	7	30	19	39	1	5 (3)	9 (4)	5 (5)	13 (1)	13 (3)	
<i>P. haemolytica</i> biotype T	9	6§	67	8	36	2	3	2	3 (2)	—	1 (1)	
<i>P. multocida</i> matrix biotype 1	25	21	84	24	32	0	22 (1)	3 (3)	—	—	—	
<i>P. multocida</i> matrix biotype 2	4	4§	100	3	44	0	4	—	—	—	—	
<i>P. pneumotropica</i>	6	5	83	6	39	0	5 (1)	1	—	—	—	
<i>P. ureae</i>	13	12	92	10	42	0	13 (1)	—	—	—	—	
<i>Plesiomonas shigelloides</i>	6	6	100	5	41	4	6	—	—	—	—	
<i>Proteus mirabilis</i>	40	40	100	22	40	2	37	3	—	—	—	
<i>P. morganii</i>	19	18	95	7	43	2	18	1 (1)	—	—	—	
<i>P. rettgeri</i>	12	12	100	10	37	2	11	1	—	—	—	
<i>P. vulgaris</i>	9	9	100	6	40	2	9	—	—	—	—	
<i>Providencia alcalifaciens</i>	27	27	100	23	40	2	20	6	1	—	—	
<i>P. stuartii</i>	3	3	100	3	39	2	2	—	—	—	1	

Table 3. (cont.)

Taxon	No. of strains (1)	Correctly identified by computer (2)		No. of bio-patterns (3)	No. of constant tests in nearest matrix* taxon† (4)	Sepa- ration from (5)	Distribution of strains by the number of their test results which differ from the matrix‡ (6)					
		No.	%				0	1	2	3	≥ 4	
R.M. bacterium	3	3	100	3	40	1	3	—	—	—	—	
<i>Salmonella choleraesuis</i>	2	2	100	2	44	2	2	—	—	—	—	
<i>S. ferlac</i>	1	1	100	1	49	2	1	—	—	—	—	
<i>S. gallinarum</i>	5	5	100	5	41	0	4	1	—	—	—	
<i>S. paratyphi</i> A	2	2	100	1	48	0	2	—	—	—	—	
<i>S. pullorum</i>	5	4	80	4	43	0	4	1 (1)	—	—	—	
<i>Salmonella</i> subgenus I	24	20§	83	18	47	1	12	8	—	1 (1)	3 (3)	
<i>Salmonella</i> subgenus II	10	8§	80	9	46	1	7 (1)	3 (1)	—	—	—	
<i>Salmonella</i> subgenus III = <i>Arizona</i>	8	7§	87	5	45	1	7	1 (1)	—	—	—	
<i>Salmonella</i> subgenus IV	7	7	100	6	46	1	6	1	—	—	—	
<i>Salmonella typhi</i>	7	6	86	6	42	0	4	1	2 (1)	—	—	
Serratia matrix biotype 1	12	11	92	12	35	0	9	1	—	1	1 (1)	
Serratia matrix biotype 2	3	3	100	3	42	3	3	—	—	—	—	
<i>Shigella sonnei</i>	7	7	100	6	44	0	6	1	—	—	—	
<i>Shigella</i> — other serotypes	34	27	79	30	34	0	32 (6)	2 (1)	—	—	—	
<i>Vibrio cholerae</i>	10	9	90	10	36	2	8	2 (1)	—	—	—	
<i>V. parahaemolyticus</i>	2	2	100	2	43	2	—	—	1	—	1	
<i>Yersinia enterocolitica</i>	7	7	100	7	39	2	6	1	—	—	—	
<i>Y. pestis</i>	12	12	100	6	42	0	11	1	—	—	—	
<i>Y. pseudotuberculosis</i>	24	24	100	11	41	1	22	2	—	—	—	
Total	827	751	90.8									

* A constant test is one with a matrix entry of 0.99 or 0.01 for the taxon in question.

† The separation between two taxa is the number of tests with matrix entries of 0.99 in one taxon and 0.01 in the other.

‡ A test result differs from the matrix if the result is +ve and the matrix entry for the taxon to which the strain belongs is 0.01 or the result is -ve and the matrix entry is 0.99. Figures in parentheses in column (6) are the number of strains which did not identify.

§ Include strains identified to composite groups as follows:

Citrobacter matrix biotype 1—seven strains identified to *E. coli*/Alkalescens-Dispar.

Escherichia coli - 16 strains identified to Citrobacter matrix biotype 1/biotype 2.

Alkalescens-Dispar - all six strains identified to *E. coli*/Alkalescens-Dispar.

Klebsiella aerogenes and *K. oxytoca* - six strains identified to *K. aerogenes* and *K. oxytoca*/*K. pneumoniae*.

Pasteurella haemolytica biotype T - one strain identified to *P. haemolytica* biotype A/biotype T.

Pasteurella multocida matrix biotype 2 - all four strains identified to *P. multocida* matrix biotype 1/biotype 2.

Salmonella subgenus I - one strain identified to *Salmonella* subgenus I/*S. choleraesuis*.

Salmonella subgenus II - three strains identified to *Salmonella* subgenus II/subgenus III.

Salmonella subgenus III - four strains identified to *Salmonella* subgenus II/subgenus III.

were treated as two-state in the identification calculations, apart from the multistate characters.

Linkage of tests. Test linkage was taken into account in the calculations for certain tests (Willcox *et al.* 1973). Some tests were carried out at more than one temperature and allowance was made for growth at the different temperatures (motility at room temperature (RT) or 37 °C, methyl red test (MR) at RT and 37 °C, Voges–Proskauer test (VP) at RT and 37 °C). Gelatin liquefaction was scored as two tests – early (1 to 5 days) or overall (1 to 28 days) – and these were treated as linked. Failure to produce acid from glucose peptone water was linked with failure to produce gas. H₂S production tested both by lead acetate papers and by triple sugar iron agar were treated as linked since most strains showed correlation of the results in these tests although the biochemical pathways involved are dissimilar (Lapage & Bascomb, 1968).

The O/F test results were treated as a multistate test since a fermentative result excludes an oxidative result or failure to produce acid, and the converse also holds; alkali production was treated independently. Pigment production with the six possible colours was also treated as a multistate test (Willcox *et al.* 1973).

Construction of matrix. The first matrix, Matrix 66 (Lapage *et al.* 1973), which was completed in 1966, was drawn from laboratory records and published data, including standard textbooks. By 1968 further information was available, particularly from tests in our laboratory by standardized methods on many strains, and a new matrix, Matrix 68, was prepared and has been in use since. The sources for the data used for each taxon are described under Results. The results given in this paper were all obtained with Matrix 68, which has shown a higher success rate in all cases than Matrix 66 (Lapage *et al.* 1973). Strains tested before the compilation of Matrix 68 and previously identified with Matrix 66 were re-identified on Matrix 68.

The probability values allotted for each taxon in each test had upper and lower limits of 0.99 and 0.01 with values between, in 0.05 intervals, from 0.95 to 0.05. The matrix was kept under review as further data became available although automatic adjustments were not made in the computer as each strain was examined (Lapage *et al.* 1973). Matrix 68 is given in Tables 1 and 2 for fermentative and non-fermentative bacteria respectively.

Probabilistic identification. In principle, from the test results given by each strain, an identification score was calculated for each taxon by comparison of the result of each test performed on the unknown strain with the probabilities in the matrix (Willcox *et al.* 1973). If any score reached a level of 0.999 then the strain was considered to be identified in that taxon. These probabilities (Matrix 68) were, with minor changes, those used during the course of this study. Some of the probabilities differ from the values that would be allotted if the results of tests on strains received after 1968 were included. These should be taken into consideration in a future revision of the matrix. To publish a further revision of the matrix at this stage would be misleading as we have no knowledge of the identification rate which would be achieved with such a matrix. However, some of the values given in Tables 1 and 2 are not those which we would recommend for diagnostic usage under the specified conditions of testing.

In certain cases if the sum of the identification scores of two particular taxa exceeded 0.999 then the strain was considered to be identified in the combined taxon. The taxa concerned are given in the footnotes to Tables 3 and 4. Separation of such taxa had proved difficult in construction of the matrix and is perhaps artificial, e.g. *Escherichia coli* and the *Alkalescens-Dispar* group.

Strains. The results obtained with 1079 strains collected between 1965 and 1970 are

Table 4. Identification of non-fermentative bacteria

Taxon	No. of strains (1)	Correctly identified by computer (2)		No. of bio-patterns (3)	No. of constant tests in matrix* (4)	Separation from nearest taxon† (5)	Distribution of strains by the number of their test results which differ from the matrix‡ (6)				
		No.	%				0	1	2	3	≥ 4
<i>Acinetobacter anitratus</i>	25	24	96	14	41	1	23	2 (1)	—	—	—
<i>A. lwoffii</i>	14	5	36	8	48	0	10 (5)	2 (2)	1 (1)	—	1 (1)
<i>Alcaligenes faecalis</i>	6	38	50	6	42	0	6 (3)	—	—	—	—
<i>Bordetella bronchiseptica</i>	14	14	100	1	47	1	14	—	—	—	—
<i>B. parapertussis</i>	13	9	69	5	47	1	9 (1)	4 (3)	—	—	—
<i>Chromobacterium lividum</i>	11	11	100	8	35	1	10	1	—	—	—
<i>Comamonas percolans</i>	8	58	62	8	44	1	5 (1)	3 (2)	—	—	—
<i>Flavobacterium meningosepticum</i>	11	11	100	5	45	1	11	—	—	—	—
<i>Moraxella</i> spp.	13	9	69	11	45	0	7 (1)	5 (3)	1	—	—
<i>Pseudomonas aeruginosa</i>	39	35	90	12	46	1	32	7 (4)	—	—	—
<i>P. fluorescens</i>	8	8	100	8	39	1	8	—	—	—	—
<i>P. mallei</i>	9	7	78	6	42	1	7 (1)	2 (1)	—	—	—
<i>P. maltophilia</i>	13	11	85	12	38	0	13 (2)	—	—	—	—
<i>P. pseudomallei</i>	17	13	76	17	33	1	5	10 (2)	2 (2)	—	—
Total	201	165	82.1								

* A constant test is one with a matrix entry of 0.99 or 0.01 for the taxon in question.

† The separation between two taxa is the number of tests with matrix entries of 0.99 in one taxon and 0.01 in the other.

‡ A test result differs from the matrix if the result is +ve and the matrix entry for the taxon to which the strain belongs is 0.01 or the result is -ve and the matrix entry is 0.99. Figures in parentheses in column (6) are the number of strains which did not identify.

§ Includes one strain identified to the composite group *Alcaligenes faecalis*/*Comamonas percolans*.

reported. Of these strains, 470 were from culture collections, i.e. NCTC, NCIB, NCMB, NCPPB and ATCC,* and many others were designated strains from experts in the relevant group of bacteria. Also included in these reference strains were some of the strains isolated in our laboratory or sent to us for identification in the early stages of the project. As these were used for reference purposes in our laboratory and were in some cases checked by reference laboratories, they have been included in the reference strains.

Identification procedure. Each reference strain for identification by computer was tested afresh in all the 48 tests, identified conventionally on these results by at least two of us and then identified by the computer method. If the identification differed from the original designation the test results were compared with those available from the source of the reference strain, which often included the results of tests not used in the Computer Laboratory, e.g. morphological tests, antigenic structure or bacteriophage sensitivity. The strains were then assigned to the taxa in the matrix.

Next, the strains were identified by the computer method. The computer decision was compared with the original designation of the strain and with the conventional identification. If the computer identification was the same as the conventional then the computer identification was considered to be correct. Very rarely a strain could not be identified conventionally but the computer suggested an identification. In this case, the results given by the strain were considered, sometimes the strain was retested and if possible additional tests were carried out, e.g. serological. If the evidence was considered sufficient by conventional criteria, the strain was included in the taxon and a correct identification by the computer recorded. If there was any doubt the strain has been excluded from this series.

Successful identification by both conventional and computer methods but into different taxa occurred only once, and this rather trivial case is described under '*Salmonella*' in the Results. A failure was scored when a strain was identified by conventional means but not by the computer.

As the purpose of the present investigation was to study computer identification of reference strains, any strain which could not be identified by either conventional or computer methods has been excluded from the following account. A reference strain can of course be an intermediate or highly unusual strain but comparison of two failures in identification was not relevant. Intermediate strains, strains which fail to identify and field strains are discussed in Lapage *et al.* (1970, 1973).

Of the 1079 reference strains which could be identified conventionally, 1028 belonged to taxa included in the matrix, while 51 were strains of taxa not included in the matrix and were tested to see if they identified in any taxon in the matrix. They were chosen from taxa, *Agrobacterium*, *Rhizobium* and *Xanthomonas*, which might occur in human specimens via plants; from taxa related to those in the matrix, *Pseudomonas* spp., *Vibrio* spp. other than *V. cholerae*, and *Zoogloea*; and from taxa likely to cause confusion, *Neisseria*.

RESULTS

Identification of strains of taxa in the matrix. The success of computer identification of the various taxa is given in Tables 3 and 4 for fermentative and non-fermentative bacteria respectively.

The first column gives the number of strains examined, the second the number of strains,

* NCTC, National Collection of Type Cultures; NCIB, National Collection of Industrial Bacteria; NCMB, National Collection of Marine Bacteria; NCPPB, National Collection of Plant Pathogenic Bacteria; ATCC, American Type Culture Collection.

followed by the percentage, which were correctly identified by the computer method. The totals show that 90.8 % of the strains of fermentative bacteria and 82.1 % of non-fermentative bacteria were correctly identified. In the case of 30 (54 %) of the 56 fermentative taxa, all the strains in the taxon concerned were identified and 90 % or over of the strains were correctly identified in a further 11 (20 %) taxa. In only four (29 %) of the 14 non-fermentative taxa were all the strains identified and in only two (14 %) were 90 % or over identified.

Column 3 of Tables 3 and 4 gives the number of strains of each taxon which gave a different pattern of results in the 57 tests carried out, i.e. the number of biopatterns, and, column 4, the number of matrix entries of 0.99 or 0.01 for each taxon. These two indices express some measure of the homogeneity of the taxon concerned, although in some taxa the number of strains tested are too few to draw any conclusions about the number of biopatterns compared to the number of strains. However, the results obtained with taxa which contained more than 20 strains were analysed and no obvious correlation was found between the identification rate and either the number of biopatterns or the number of matrix entries of 0.99 and 0.01.

The number of test differences of 0.99 and 0.01 of each taxon from its nearest neighbour (column 5 of Tables 3 and 4) represents the minimum number of such tests available for its differentiation. If the taxa are taken individually the number of 0.99/0.01 separating tests is not a complete guide as to how well strains of the taxon will identify since some taxa with no 0.99/0.01 separating tests gave 100 % identification rates, whereas some taxa with two 0.99/0.01 separating tests did not. However, at least two 0.99/0.01 separating tests are required for all taxa before a good identification rate can be assumed. The difference in identification rate for strains of taxa with less than two and strains of taxa with two or more separating tests is significant by the Chi-squared test ($P < 0.005$).

The distribution of the 112 strains which failed to identify among those showing nil, one, two, three or four or more differences from matrix entries of 0.99 or 0.01 is shown in column 6 of Tables 3 and 4. The percentage of these strains which did not identify was in these five categories respectively 4.6 % (no difference), 30 % (one difference), 55 % (two differences), 45 % (three differences) and 69 % (four or more differences). Clearly, as would be expected, the number of differences from definitive matrix entries does play a considerable part.

Separate taxa

References are given for each taxon to many of the reports consulted during the initial compilation of the matrix and its modifications. However, not every reference consulted can be given because in the early stages of the compilation a wide variety of sources was used and some data obtained by personal communication and adequate records were not kept of all the sources. As well as the references quoted the standard textbooks were consulted in most cases (in particular Breed, Murray & Smith, 1957; Prévot, 1961; Edwards & Ewing, 1962; Wilson & Miles, 1964; Cowan & Steel, 1965; Kauffmann, 1966).

Acinetobacter. (Brooke, 1951a; Brisou & Prévot, 1954; Klinge, 1959; Courtieu, Chassignol & Longeray, 1961; Cetin, 1962; Hendrie, Hodgkiss & Shewan, 1964; Steel & Cowan, 1964; Gilardi, 1967; Thornley, 1967; Pintér & Bende, 1968.) Two species were included – *Acinetobacter anitratus* and *A. lwoffii*. Strains of the species of *Achromobacter* described by Mannheim & Stenzel (1962) were included in *A. anitratus* or *A. lwoffii*. Of the 25 strains of *A. anitratus*, 24 (96 %) were correctly identified. *Acinetobacter anitratus* had the highest identification score for the remaining strain but the score for *Pseudomonas mallei* prevented identification at the level of 0.999. Only five (36 %) of the 14 strains of *A. lwoffii* were cor-

rectly identified and although the highest scoring taxon in each case was *A. lwoffii*, identification was prevented by the scores for *Pseudomonas mallei* and *Bordetella parapertussis*.

Actinobacillus. (Tunncliffe, 1941; Taylor, 1944; Ristic, Herzberg, Sanders & Williams, 1956; Phillips, 1960, 1961; Vallée, Thibault & Second, 1963.) Two species were included – *Actinobacillus equuli* and *A. lignieresii*. Of the 13 strains of *A. lignieresii*, 10 (77 %) identified correctly. With the other three strains, although *A. lignieresii* was the highest scoring taxon, identification was prevented by the scores for *A. equuli*. Of the seven strains of *A. equuli*, six (86 %) were identified correctly.

Aeromonas. (Griffin, Snieszko & Friddle, 1953; Crawford, 1954; Pivnick & Sabina, 1957; Eddy, 1960, 1962; Schubert, 1960, 1961, 1962, 1963, 1964, 1967; Ewing, Hugh & Johnson, 1961; Park, 1962; Eddy & Carpenter, 1964; Catsaras & Buttiaux, 1965; Bain & Shewan, 1968.) Three species were included – *Aeromonas hydrophila*, *A. formicans* and *A. salmonicida* (*A. shigelloides* is considered under *Plesiomonas shigelloides*). Of the 16 strains of *A. hydrophila*, 15 (94 %) were correctly identified and of the 11 strains of *A. formicans* ten (91 %) were also correctly identified. One strain of each species failed to identify due to the score reached for the other and these were probably intermediate strains. All the 12 strains of *A. salmonicida*, a very homogeneous species, were correctly identified.

Alcaligenes and *Comamonas*. (Nyberg, 1935; Conn, 1942; Ulrich & Needham, 1953; Galarneault & Leifson, 1956; Moore & Pickett, 1960; Hugh & Ryschenkow, 1961; Davis & Park, 1962; Park, 1962; Koontz & Faber, 1963; Richter & Kress, 1967.) Two species were included – *Alcaligenes faecalis* and *Comamonas percolans*. Both in the compilation of the data on these taxa and in the identification in the matrix it was difficult to differentiate these two species, particularly as the matrix did not include investigation of the arrangement of the flagella.

In Matrix 66, *Alcaligenes faecalis* and *Comamonas percolans* were placed in two separate taxa. They were later combined but the combined taxon prevented identification of strains of other taxa. It was therefore decided to keep them separate but allow identification in a combined taxon if the sum of the scores of the two taxa exceeded the identification level of 0.999. The only test in our matrix which provided an 0.99/0.01 separation between the two taxa was growth in KCN medium. Only two (33 %) of the six strains of *A. faecalis* identified correctly, and of the remaining four only one (17 %) identified in a combined *A. faecalis*/*C. percolans* taxon. Identification of the other three strains was prevented by the scores for *Pseudomonas maltophilia* and *Bordetella bronchiseptica*. Of the eight strains of *C. percolans*, four (50 %) were identified correctly and one identified in the combined group taxon. With the three remaining strains identification was prevented by the scores for *P. maltophilia* and *B. bronchiseptica*.

This group of motile organisms, which give few positive results in the usual diagnostic tests, are not easy to differentiate, and included in *Alcaligenes faecalis* were strains of *A. faecalis*, *A. faecalis* var. *odorans*, *A. viscosus* and other strains. In *Comamonas percolans* were included strains of *C. percolans*, *Vibrio alcaligenes*, *Pseudomonas alcaligenes*, *P. pseudoalcaligenes*, and other strains. This was done for convenience since the species in each group could not be differentiated on the tests in our matrix. The taxonomic relationships of these organisms appear uncertain and further study is needed.

Bordetella. (Lautrop, 1960.) Two species were included – *Bordetella bronchiseptica* and *B. parapertussis*. *Bordetella pertussis* was not included because strains do not in general grow on nutrient agar. All of the 14 strains of *B. bronchiseptica* were correctly identified and appear to form a coherent group. Of the 13 strains of *B. parapertussis*, nine (69 %) were

correctly identified but with four strains identification was prevented by the scores for *Acinetobacter lwoffii*.

Chromobacterium. (Sneath, 1956; Moffett & Colwell, 1968.) The psychrophilic and mesophilic violet-pigmented species *Chromobacterium lividum* and *C. violaceum* were included. Eleven strains of *C. lividum* were identified correctly, one of these strains was originally designated *C. amethystinum*. Of the 14 strains of *C. violaceum*, 12 (86 %) were correctly identified. The other two showed three and four differences from the matrix entries of 0.99 and 0.01 and failed to identify although *C. violaceum* was the taxon with the highest identification score.

Citrobacter. (Werkman & Gillen, 1932; Stuart, Wheeler, Rustigian & Zimmerman, 1943; Davis & Ewing, 1966.) The genus was divided in our matrix into two taxa – matrix biotype 1 and matrix biotype 2. Matrix biotype 1 is equivalent to typical strains of *Citrobacter freundii*. In Matrix 66 only one species, *C. freundii*, was included and strains classed as irregular enterobacteria were identified by the computer as *Citrobacter freundii* although they differed in some tests. These strains were considered to resemble *Citrobacter* more than any other group and included in the matrix as *Citrobacter* matrix biotype 2. They have been independently observed and described by Macierewicz (1966), who named them *Padlewskia*, by Frederiksen (1970), who named them *C. koseri*, and by Young, Kenton, Hobbs & Moody (1971), who named them *Levinea*. Of the 61 strains belonging to *Citrobacter* matrix biotype 1, 50 (82 %) identified correctly and a further seven (11 %) identified when the combined score for *Citrobacter* matrix biotype 1 and matrix biotype 2 was used. Of the 20 strains of *Citrobacter* matrix biotype 2, 19 (95 %) identified correctly. One strain of *C. ballerupensis* whose reactions resembled those of a strain of *Salmonella* failed to identify although *Citrobacter* matrix biotype 1 was the highest identification score with *Salmonella* subgenus I as the next.

Comamonas. See *Alcaligenes* and *Comamonas* above.

Edwardsiella. (Ewing, McWhorter, Escobar & Lubin, 1965; Ewing, McWhorter, Ball & Bartes, 1967; Sakazaki, 1967; Bhat, Myers & Carpenter, 1967.) One species, *Edwardsiella tarda*, was included and all nine strains were correctly identified.

Enterobacter. (Brooke, 1951b, 1953; Report, 1958; Grimes, 1961; Report, 1963; Fife, Ewing & Davis, 1965.) Three species were included: *Enterobacter aerogenes*, *E. cloacae* and *E. liquefaciens*. The classification of *Enterobacter* and *Klebsiella* and the choice of characteristics and taxa for use in the matrix proved difficult and a numerical taxonomy was undertaken (Bascomb *et al.* 1971). The results of this study were used to improve the matrix and all 3 strains of *E. aerogenes*, 34 of *E. cloacae* and five of *E. liquefaciens* were correctly identified.

Erwinia. (Cruickshank, 1935; Jøker, Nørholm & Siboni, 1965; Muraschi, Friend & Bolles, 1965; Graham & Hodgkiss, 1967.) In Matrix 66 two taxa were included: *Chromobacterium typhiflavum* and *Enterobacter* 'pigmenté' (Leclerc, 1962). However, the reactions given by the strains of these taxa were found to be similar and the two taxa were combined under the name *Erwinia herbicola*, of which Graham & Hodgkiss (1967) had shown *C. typhiflavum* to be a junior synonym. A separate study was made of this group and additional strains included to make a total of 32: *C. typhiflavum* (5), *Enterobacter* 'pigmenté' (10), *Erwinia herbicola* (10), *E. lathyri* (2), *E. ananas* (2), *E. milletiae* (1) and *E. uredovora* (2). Many of these 32 strains were from plant sources but 26 (81 %) were identified, as *E. herbicola*, despite differences in their results of three, four and five from the matrix entries of 0.99 or 0.01. Of the six strains which failed to identify, in one case *Citrobacter* biotype 2 reached the highest identification score and in another *Enterobacter cloacae*. Possibly

these strains belonged to these two species. Yellow pigmented strains of *Citrobacter* have been described by Thomas & Elson (1957) and several yellow pigmented strains of *E. cloacae* are kept in the NCTC.

Escherichia adecarboxylata. (Leclerc, 1962.) All the seven strains of this homogeneous species identified correctly. Some strains were non-pigmented and others continuously produced non-pigmented mutants. The exact generic position of this species is not certain.

Escherichia coli and the *Alkalescens-Dispar* group. Two taxa were included in the matrix: *Escherichia coli* and the *Alkalescens-Dispar* group, which may be regarded as part of the species *E. coli*. Strains which were either non-motile, anaerogenic or ONPG and lactose-negative usually did not reach identification level in either group but required a combined score for *E. coli* and the *Alkalescens-Dispar* group. Combination of these two groups in the matrix as one taxon, however, led to difficulty in their identification because the scores for *Shigella* became too high to permit identification. The two groups were therefore kept separate in Matrix 68 but identification was considered correct if the combined score for the two reached the required level of 0.999. Of the 60 strains of *E. coli*, 42 (70 %) identified as *E. coli* while 16 (27 %) identified when the identification score for the combined *E. coli/Alkalescens-Dispar* group was considered, so that a total of 58 strains (97 %) could be identified. Of the 11 strains of the *Alkalescens-Dispar* group, six (55 %) could be identified by the combined *E. coli/Alkalescens-Dispar* group scores. Two strains of *E. coli*, two strains of the *Alkalescens-Dispar* group and three strains belonging to the group but not typical of either taxon (recorded in the *Alkalescens-Dispar* group in Table 3) could not be identified by the computer method as identification scores in *Shigella* reached a level which prevented identification.

Flavobacterium. (Brody, Moore & King, 1958; King, 1959; Cabrera & Davis, 1961; Sugathadasa & Arseculeratne, 1963; Nelson & Shelton, 1965.) Only one species, *Flavobacterium meningosepticum*, was included and all of the 11 strains were identified correctly.

Hafnia. (Sakazaki, 1961; Fife *et al.* 1965; Carpenter, Lapage & Steel, 1966.) One species, *Hafnia alvei*, was included. Of the 18 strains of *H. alvei*, 17 (94 %) were correctly identified and one strain did not identify though the highest score was *H. alvei*.

Klebsiella. (Brooke, 1951*b*; Lautrop, 1956; Cowan, Steel, Shaw & Duguid, 1960; Fife *et al.* 1965; Slopek & Durlakowa, 1967; Kaluzewski, 1967.) Difficulty was experienced in the identification of strains of this genus. In Matrix 66 we included the taxa in the classification of Cowan *et al.* (1960); however, we were unable to identify the majority of the strains. A numerical taxonomy study of 177 strains of the *Klebsiella* and related groups (Bascomb *et al.* 1971) was carried out and the taxa used in Matrix 68 were as follows: *Klebsiella aerogenes* and *K. oxytoca*; *K. pneumoniae*; *K. ozaenae*; *K. rhinoscleromatis*; and an unnamed group of *Klebsiella* whose characteristics were described by Bascomb *et al.* (1971). Of the 89 strains of the combined *K. aerogenes* and *K. oxytoca* group (which includes *K. edwardsii* and its two varieties), 78 (88 %) were identified correctly and a further six (7 %) were identified if the identification scores for the combined *K. aerogenes* and *K. oxytoca* group were added to those for *K. pneumoniae*. The remaining five strains all had the combined *K. aerogenes* and *K. oxytoca* group as the highest score and as the next highest one of the other *Klebsiella* groups. All of the six strains of *K. ozaenae*, five strains of *K. pneumoniae*, six strains of *K. rhinoscleromatis* and four strains of the unnamed *Klebsiella* group were identified correctly.

Kluyvera. (Asai, Okumura & Tsunoda, 1957; Eddy, 1960; Asai, Iizuka & Komagata, 1962.) Asai *et al.* (1962) have suggested that this genus should be included in the genus *Escherichia*. However, the genus appeared to constitute a separate taxon as the three avail-

able strains were identified correctly in a taxon for *Kluyvera* included in the matrix, and the identification scores for *Escherichia coli*, the *Alkaescens-Dispar* group, or *Citrobacter* did not prevent identification.

Moraxella. (Henriksen, 1960, 1963; Bøvre & Henriksen, 1962; Piéchaud, 1963; Bijsterveld, 1967.) Sufficient suitable tests were not included in the matrix to separate the species of the genus *Moraxella* adequately, therefore a combined taxon of all *Moraxella* species was included. Of a total of 13 strains of *Moraxella*, nine (69 %) were correctly identified into this taxon: two of three strains of *M. liquefaciens*, two of three strains of *M. bovis*, two strains of *M. nonliquefaciens*, two of *M. lacunata*, and one of *M. osloensis*. In addition to the two strains of the above species which did not identify, one strain each of *M. phenylpyruvica* and *M. kingii* did not identify. Identification was only moderately successful in this taxon and was prevented in some cases by the scores for *Acinetobacter lwoffii*.

Pasteurella. (Stableforth & Galloway, 1959; Steel & Midgley, 1962; Smith & Thal, 1965.) Four taxa were included at first – *Pasteurella multocida* (Jones, 1921; Smith, 1958; Talbot & Sneath, 1960; Watt, 1965), *P. haemolytica* (Newsom & Cross, 1932; Biberstein, Myer & Kennedy, 1958; Biberstein, Gills & Knight, 1960; Smith, 1961; Boháček & Mráz, 1967), *P. pneumotropica* (Jawetz, 1950) and *P. ureae* (Henriksen & Jysum, 1960, 1961; Henriksen, 1961, 1962; Omland & Henriksen, 1961; Jones, 1962; Jones & O'Connor, 1962; Heyl, 1963; Wang & Haiby, 1966) – and information on two new unnamed species was obtained from Hoag, Wetmore, Rogers & Meier (1962) and Brodie & Henderson (1963).

Strains of this group were difficult to identify and identification was more successful when *Pasteurella multocida* was divided into two biotypes and *P. haemolytica* into biotype and biotype T. Of the 13 strains of *P. ureae*, 12 (92 %) were identified correctly and one failed to identify. Of the six strains of *P. pneumotropica*, five (83 %) were identified correctly, confirming the separation of this taxon from *P. multocida*. Atypical strains of *P. multocida* proved difficult to identify though several experimental matrices were tested, finally the typical *P. multocida* strains were included as matrix biotype 1 and strains (more commonly found from dog bites) which failed to grow on MacConkey's agar, and usually did not produce acid from any sugar other than glucose and sucrose, formed matrix biotype 2. With these two biotypes in the matrix, 21 (84 %) of the 25 strains of *P. multocida* matrix biotype 1 were identified correctly. The remaining four strains failed to identify and were not identified into a combined *P. multocida* matrix biotype 1/biotype 2 taxon. None of the four strains of matrix biotype 2 were identified into this taxon – however, they did identify into a combined *P. multocida* matrix biotype 1/biotype 2 taxon. None the less the taxon was retained as the overall identification of strains of *P. multocida* was more successful than if the biotypes were combined. Of 23 strains of *P. haemolytica* biotype A, seven (30 %) identified correctly and of nine strains of *P. haemolytica* biotype T, five (56 %) identified correctly while one strain identified in a combined *P. haemolytica* biotype A/biotype T taxon. These identification rates were unsatisfactory, largely due to the number of biopatterns obtained and the large number of strains with two, three, four or five test differences from the matrix entries of 0.99 and 0.01. A numerical taxonomy study of a large number of *Pasteurella* strains is needed.

Plesiomonas. (Ewing *et al.* 1961; Habs & Schubert, 1962; Eddy & Carpenter, 1964.) One species was included in this genus, *Plesiomonas shigelloides*, and all six strains of this homogeneous taxon were correctly identified.

Proteus and Providencia. (Stuart, Wheeler & McGann, 1946; Singer & Bar-Chay, 1954; Shaw & Clarke, 1955; Ewing, Suassuna & Suassuna, 1960; Sen, 1962; Richard, 1965, 1966; Prakash, Dayal & Kalra, 1966; Giammanco, 1966; Rauss & Vörös, 1967.) Six species

were included: *Proteus mirabilis*, *P. morganii*, *P. rettgeri*, *P. vulgaris*, *Providencia alcalifaciens* and *Providencia stuartii* (corresponding to *Providencia* biotypes A and B respectively). Little difficulty was experienced in the identification of strains of these genera. All the 40 strains of *P. mirabilis*, 12 of *P. rettgeri*, 9 of *P. vulgaris*, 27 of *Providencia alcalifaciens* and 3 of *Providencia stuartii* were identified correctly. Of 19 strains of *P. morganii*, 18 (95 %) were identified correctly and the other strain failed to reach identification level due to the identification score for *Providencia alcalifaciens*.

Pseudomonas. (Rhodes, 1959; Hugh & Ryschenkow, 1961; Park, 1962; Hugh & Leifson, 1963; Colwell, 1964; Wahba & Darrell, 1965; Redfearn, Palleroni & Stanier, 1966; Stanier, Palleroni & Doudoroff, 1966; Weaver, 1967; Lapage, Hill & Reeve, 1968.) *Pseudomonas aeruginosa*, *P. fluorescens*, *P. mallei*, *P. maltophilia* and *P. pseudomallei* were included as separate taxa. Other species of *Pseudomonas* were not included as the tests in the matrix were not suitable for their differentiation. Of the 39 strains of *P. aeruginosa*, 35 (90 %) were correctly identified and the remaining strains failed to identify due to the identification scores reached by *P. fluorescens*. All the eight strains of *P. fluorescens* were correctly identified, and of the nine strains of *P. mallei*, seven (78 %) were correctly identified and two failed to identify due to the scores reached by *Moraxella*. Of the 13 strains of *P. maltophilia*, 11 (85 %) were correctly identified and, with the other two strains, identification was prevented in one case by the score for *Comamonas* and in the other for *Acinetobacter lwoffii*. Of the 17 strains of *P. pseudomallei*, 13 (76 %) were correctly identified and the other four were not, owing to the scores reached by *P. fluorescens*. The tests used in the matrix are not entirely satisfactory for the differentiation of even these *Pseudomonas* spp.

R. M. Bacterium. (Ross *et al.* 1966.) This unclassified enterobacterium, isolated from red mouth disease of trout, was included in the matrix since the distribution of the organism was unknown. All three strains of the taxon were identified correctly.

Salmonella. (Hinshaw, Browne & Taylor, 1943; Blaxland, Sojka & Smither, 1956; Kauffmann, 1961, 1965, 1966; Costin *et al.*, 1964; Ewing & Ball, 1966; Ewing & Fife, 1966.) Included as taxa in the matrix were the four *Salmonella* subgenera I, II, III (= *Arizona*) and IV. In addition, *S. choleraesuis*, *S. gallinarum*, *S. ferlac*, *S. paratyphi* A, *S. pullorum* and *S. typhi* were included as separate taxa. The two strains of *S. choleraesuis*, all five strains of *S. gallinarum*, the one strain of *S. ferlac*, both strains of *S. paratyphi* A and all seven strains of subgenus IV were correctly identified. Only 1 of the 5 strains of *S. pullorum* failed to identify due to the score reached by *S. gallinarum*. Of the seven strains of *S. typhi*, six (86 %) identified correctly, including a lactose-positive strain which was included in the series. The strain which did not identify differed biochemically in four tests from a typical strain of *S. typhi*, but was assumed to be a strain of *S. typhi*, as it had the antigenic structure Rough, Vi:d.

Of the 24 strains of *Salmonella* subgenus I, 20 (83 %) were identified correctly. A strain of *Salmonella thompson*, a species which belongs to subgenus I, was atypical in several characters and failed to identify. Two other strains of subgenus I, *S. sendai* and *S. typhisuis*, also failed to identify. These two species, though classified in *Salmonella* subgenus I (Kauffmann, 1961), have rather different characteristics from those typical of the subgenus. A strain of *S. abortusovis* identified as *S. choleraesuis* – a species also classified in subgenus I but entered as a separate taxon in Matrix 68. *Salmonella* species are, however, recognized largely on serological rather than biochemical grounds and it would be difficult, if not impractical, to add every aberrant serotype as a different taxon. This identification of the strain of *S. abortusovis* into *S. choleraesuis* is the only instance in the whole series of strains in which a strain of a taxon in the matrix identified in the incorrect taxon; it has been scored

as not identified in Table 3. Subgenera II and III presented more difficulty as five of ten strains in subgenus II identified in that taxon and a further three were identified in a combined group of subgenera II and III. Similarly, three of the eight strains of subgenus III identified in that taxon and a further four were identified in the combined group. A strain of *S. basel* (a serotype classified in subgenus II) failed to identify in subgenus II or in a combined subgenus II and III as identification was prevented by subgenus I. A strain of *S. windhoek* (which belongs to subgenus II) also failed to identify, though subgenus II reached the highest identification score and subgenus IV the next highest, as might be expected since this strain of *S. windhoek* grew in KCN medium – a characteristic of subgenus IV. The remaining strain of subgenus III which did not identify in that taxon or in combined subgenera II and III had the highest identification score for subgenus III though identification level was not reached.

There does not appear to be a great distinction between subgenera II and III and it has been suggested that they should be combined. A numerical taxonomy study, based on both biochemical and serological data, of the genus *Salmonella* is needed to support the validity of the subgenera and to establish which species may form further clusters. Many atypical strains are placed into the subgenera and the value of the subgenera as a primary distinction needs to be established; more than four subgenera may be required, particularly if they are lowered in rank to *Salmonella* species, relegating the present species to serotypes (Le Minor, Rohde & Taylor, 1970).

Serratia. (Ewing, Davis & Reavis, 1959; Martinec & Kocur, 1961; Ewing, Davis & Johnson, 1962; Ewing, Johnson & Davis, 1962; Fife *et al.* 1965; Sedlák, Dlabac & Motlíková, 1965.) Two taxa were included – *Serratia* matrix biotype 1, which corresponded to *Serratia marcescens*, and *Serratia* matrix biotype 2, which has been described in Bascomb *et al.* (1971) and resembles phenon B of Grimont (1969). These strains of *Serratia* were negative in ornithine decarboxylase, produced acid from arabinose, lactose, raffinose and xylose and were anaerogenic. They differ from the description of *S. marcescens* var. *kiliensis* (Fife *et al.* 1965) in producing acetoin at 37 °C and room temperature and by producing acid from arabinose and raffinose. Of the 12 strains of *Serratia* matrix biotype 1, 11 (92 %) were correctly identified as were the three strains of *Serratia* matrix biotype 2. The failure of one strain of *Serratia* matrix biotype 1 to identify was caused by the score for *Enterobacter liquefaciens*. It has been suggested that *S. marcescens* and *E. liquefaciens* are closely related (Hamon, Le Minor & Peron, 1970; Bascomb *et al.* 1971).

Shigella. (Carpenter *et al.* 1966; Adler & King, 1967; Carpenter, 1968.) Great difficulty was experienced in making suitable taxa for use in the matrix in the identification of *Shigella* species, and a number of experimental matrices were tested, even one which included the biochemical reactions of all the described serotypes of the species *Shigella dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. This complete matrix did not prove satisfactory as there were insufficient biochemical differences between the serotypes to enable identification to be reached. Many strains of particular serotypes are biochemically atypical. For identification it was found most satisfactory to use only two taxa, *Shigella sonnei* and ‘*Shigella* – other serotypes’.

All the seven strains of *Shigella sonnei* were correctly identified, while only 27 (79 %) of the 34 strains of other serotypes could be correctly identified. Of the ten strains of *S. dysenteriae*, nine (90 %) identified correctly, while the identification score for *S. sonnei* prevented identification of the remaining strain. Of 11 strains of *S. boydii*, six (54.5 %) identified correctly and the identification of the other five was prevented by the score for the Alkaliscens-Dispar group. Of 13 strains of *S. flexneri*, 12 (92 %) identified correctly and one

strain failed to identify due to the score for *Salmonella gallinarum*. In all these cases the score for 'Shigella – other serotypes' was the highest.

A numerical taxonomy study of a large number of *Shigella* strains seems to be required to define the biochemical clusters in the genus *Shigella*. Until such a study has been made it is not certain that *Shigella* strains other than *S. sonnei* can be clearly separated from *Escherichia coli* by numerical identification, therefore such a study should include members of *E. coli* and the *Alkaescens-Dispar* group.

Vibrio. (Pollitzer, 1955; Burrows & Pollitzer, 1958; Davis & Park, 1962; Fujino *et al.* 1965; Carpenter, 1966; Ewing, Davis & Martin, 1966; Sakazaki, Gomez & Sebald, 1967; Baross & Liston, 1968.) Two taxa were included – *Vibrio cholerae* together with other related vibrios such as the El Tor biotype and the so-called non-agglutinable (NAG) vibrios – and as a separate taxon *V. parahaemolyticus*. Organisms previously classified as *Vibrio*, such as *V. fetus*, were considered to belong to the genus *Campylobacter* (Sebald & Véron, 1963), but as they grow poorly, if at all, on nutrient agar they were not included in this study. Other marine vibrios apart from *V. parahaemolyticus* were not included. Of the ten strains of *V. cholerae*, nine (90 %) were correctly identified and failure of identification of the one strain was due to the score reached by *V. parahaemolyticus*. Both strains of *V. parahaemolyticus* were correctly identified.

Yersinia. (Chen, 1949; Pollitzer, 1954; Daniëls, 1963; Daniëls & Goudzwaard, 1963; Knapp & Thal, 1963; Mollaret, 1966, 1967; Winblad, Niléhn & Jonsson, 1966; Niléhn, 1967; Jansson, Wallgren & Ahvonen, 1968.) Three species were included – *Yersinia pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. All seven strains of *Y. enterocolitica*, 12 of *Y. pestis* and 24 of *Y. pseudotuberculosis* were identified correctly.

Strains of taxa not in the matrix. The results obtained with these 51 strains are given in Table 5 and all but 11 (21.6 %) failed to identify. For those strains which identified, the number of test results differing from the matrix entries of 0.99 or 0.01 for the taxon into which they identified are shown in Table 5. One of 11 strains of *Agrobacterium tumefaciens* differed from the others in a number of characters and identified as *Flavobacterium meningosepticum*, which it resembled in all but two characters. Three of four strains of *Neisseria catarrhalis* and one of *N. cinerea* showed no test differences from the matrix entries of 0.99 or 0.01 for *Moraxella* and identified in that taxon. Two strains of *Neisseria pharyngis* identified as *Pasteurella ureae* and showed only one and two test result differences from the matrix entries of 0.99 or 0.01 for that taxon. This shows only that they resemble each other in the tests in the matrix and that the less reactive Parvobacteriaceae may be confused with *Neisseria*.

Since only a limited number of taxa of *Pseudomonas* (*P. aeruginosa*, *P. fluorescens*, *P. mallei*, *P. maltophilia*, and *P. pseudomallei*) were included in the matrix, a number of strains of other species of *Pseudomonas* (Table 5) were tested. Two of the three strains of *P. stutzeri* showed no test differences from *P. fluorescens* in the tests in the matrix and identified in that taxon. However, all the strains of *Pseudomonas* belonging to a species not in the matrix and which failed to identify had as their highest identification score one of the taxa of *Pseudomonas* present in the matrix. Identification by our method of species of this genus requires further study and a further matrix. One strain of *Rhizobium meliloti* which showed no test differences with the 0.99 or 0.01 entries for *P. fluorescens* identified in this species. This is not unexpected as suitable tests for their differentiation were not included.

Since only taxa for *Vibrio cholerae* and *V. parahaemolyticus* had been included in the matrix, one strain of each of four other *Vibrio* species were tested (Table 5). One – *V. anguillarum* – identified as *Aeromonas hydrophila*. One strain each of *Xanthomonas campestris*,

Table 5. Identification of strains of taxa not in the matrix

Taxon	No. of strains	Strains identified by the computer		
		No.	Taxon to which identified	Test differences*
<i>Agrobacterium tumefaciens</i>	11	1	<i>Flavobacterium meningosepticum</i>	2
<i>Neisseria animalis</i>	1	—	—	—
<i>N. canis</i>	1	—	—	—
<i>N. catarrhalis</i>	4	3	<i>Moraxella</i> spp.	0
<i>N. caviae</i>	1	—	—	—
<i>N. cinerea</i>	1	1	<i>Moraxella</i> spp.	0
<i>N. cuniculi</i>	1	—	—	—
<i>N. denitrificans</i>	1	—	—	—
<i>N. flavescens</i>	2	—	—	—
<i>N. pharyngis</i>	2	2	<i>Pasteurella ureae</i>	1, 2
<i>Pseudomonas cepacia</i>	4	—	—	—
<i>P. chlororaphis</i>	1	—	—	—
<i>P. diminuta</i>	1	—	—	—
<i>P. ovalis</i>	1	—	—	—
<i>P. putrefaciens</i>	6	—	—	—
<i>P. stutzeri</i>	3	2	<i>Pseudomonas fluorescens</i>	0
<i>Rhizobium japonicum</i>	1	—	—	—
<i>R. meliloti</i>	1	1	<i>Pseudomonas fluorescens</i>	0
<i>Vibrio albensis</i>	1	—	—	—
<i>V. anguillarum</i>	1	1	<i>Aeromonas hydrophila</i>	1
<i>V. fischeri</i>	1	—	—	—
<i>V. metschnikovii</i>	1	—	—	—
<i>Xanthomonas campestris</i>	1	—	—	—
<i>X. juglandis</i>	1	—	—	—
<i>X. pelargonii</i>	1	—	—	—
<i>Zoogloea ramigera</i>	1	—	—	—
Total	51	11		

* The number of tests in which the strain gave a +ve result and the matrix entry for the taxon to which it identified was 0.01 or the strain gave a -ve result and the matrix entry was 0.99.

X. juglandis and *X. pelargonii* failed to identify, which is of interest in view of the close relationship phenetically of this genus to *Pseudomonas*. A strain of *Zoogloea ramigera* – the type strain (Wisconsin strain, ATCC19623) proposed by Crabtree & McCoy (1967) – failed to identify.

DISCUSSION

The rate of success in identification varied between groups of taxa. The fermentative taxa gave the highest overall rate of identification (90.8 %) and in 54 % of these taxa all the strains were identified. The non-fermentative taxa gave an overall identification rate of only 82.1 % and only 29 % of these taxa gave an identification rate of 100 %. Of strains belonging to taxa not included in the matrix, 21.6 % were identified to one of the taxa in the matrix. But as these strains showed so few differences with the 0.99, 0.01 entries in the matrix (Table 5), addition of further tests to the matrix suitable for the differentiation of these taxa is required.

It is not clear exactly what factors operate in successful identification. The number of biopatterns given by strains in a taxon showed no correlation with success in identification in that taxon. The number of 0.99 or 0.01 differences, for a given taxon, from the taxon closest to it in the matrix does, however, play some part, as a successful identification rate cannot be assumed, though it may be obtained, unless two or more 0.99/0.01 test differences are between the taxa concerned. The difference in identification rates for fermenters and non-

fermenters can be accounted for by the lack of discriminating tests for non-fermenters; the identification rate for non-fermenters (81.6 %) does not differ significantly from those fermenters in taxa with less than two separating tests (81.8 %).

The only fully correlated factor indicated was the number of differences of the strain from the matrix entries of 0.99 or 0.01 for that taxon. Otherwise it would seem to us that there is no method *a priori* in a biological system of determining whether a probabilistic matrix will operate successfully and an empirical trial has to be made.

In some cases a combined score for two taxa is needed to reach identification, e.g. *Escherichia coli* and the *Alkalescens-Dispar* group, which are clearly closely related even if separable. The inclusion of tests to separate such groups, e.g. serological, might overload the matrix with tests inapplicable to most taxa. Moreover, for practical purposes, identification in a combined taxon may be sufficient.

It is also obvious from Table 3 that, with the exception of *Actinobacillus lignieresii*, the *Alkalescens-Dispar* group, *Pasteurella haemolytica* A and T, and 'Shigella - other serotypes', a very high rate of identification was achieved. Many of the strains of the other fermentative taxa which did not identify were either true intermediate strains, or ones which varied from the expected test results in their taxon; such differences tend to be deliberately ignored by the bacteriologist when making a decision about the identification of these strains. If the logic of such decisions could be formulated it might be possible to include it in the identification program.

Similarly, formulation of more precise definitions for taxa would enhance identification. It would remove the weighting often used in conventional identification, e.g. fermentative violet pigmented organisms are considered *Chromobacterium violaceum*, as were the two strains which failed to identify and yet had three and four test differences from the matrix figures in this study.

We wish to thank the directors of all the laboratories who sent us strains and collaborated in the project. Our thanks are also due to the Public Health Laboratory Service for help and support, and to the Department of Health and Social Security for the grant which made the work possible.

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