Recurrent Group Analysis in the Classification of Flexibacteria

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SUMMARY

A computer programme was used to group 85 strains of flexibacteria on the basis of similarity in 84 structural, physiological and biochemical characteristics. The procedure gave 19 groups, the members of which were very similar in their characteristics. The groups could be grouped in seven assemblages whose members showed lesser, but still considerable, similarity. The groups may represent taxonomic entities at the species level; the assemblages, those at the generic level.

A computer programme originally developed for identifying communities by determining recurrent groups of invertebrates* on the basis of their frequency of co-occurrence in samples of forest litter, soil, and zoo-plankton (Fager, 1957; Fager & McGowan, 1963) was used, with little change, for grouping the strains of flexibacteria investigated by Lewin & Lounsbery (1969). For each pair of strains, the programme calculated an index of affinity, the geometric mean of the proportion of common characteristics corrected for the number of characteristics recorded for the strains \( \frac{J}{\sqrt{A \times B}} - \frac{1}{2} \sqrt{B} \) where \( A \) and \( B \) are the total numbers of characteristics recorded for the two strains, \( B \geq A \), and \( J \) is the number of common characteristics, i.e. positive matches). All characteristics were weighted equally. Unlike most grouping procedures (Sneath, 1962), the programme first formed the largest group within which all possible pairs of strains had affinity indices at or above a preset value. The members of the first group were then removed from further consideration and the largest possible group was formed among the remaining strains. This process was continued until all strains were either placed in a group or designated associates of a group. The latter were strains that had affinity indices above the preset level with some, but not all, of the members of a group. Where two or more groups of the same size were possible, the programme selected the one for which the sum of affinity indices for all pairs of species within the group was greatest. When the alternative groups had species in common, this resulted in elimination of one or more groups. When there were no species in common, all groups were eventually selected. It has been found in previous work that values of the index in the range 0.500 to 0.600 give useful groupings. In the present case, the use of 0.575 gave a number of groups of reasonable size without involving very large numbers of alternative groups that would require excessive computer time.

The 84 structural, physiological and biochemical characteristics that were recorded for the strains are given in Table 1. Information on the metabolism of various carbo-
Table 1. Characteristics recorded, based on the data of Lewin & Lounsberg (1969).

<table>
<thead>
<tr>
<th>Guanine + cytosine mole % in DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 30 %</td>
</tr>
<tr>
<td>30.0 to 32.5</td>
</tr>
<tr>
<td>32.6 to 35.0</td>
</tr>
<tr>
<td>35.1 to 37.5</td>
</tr>
<tr>
<td>Greater than 50.0</td>
</tr>
</tbody>
</table>

Morphology, etc.

<table>
<thead>
<tr>
<th>Length</th>
<th>Helical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 10 μ in length</td>
<td>Not helical</td>
</tr>
<tr>
<td>10 to 50 μ in length</td>
<td>Rhapidosomes observed</td>
</tr>
<tr>
<td>Greater than 50 μ in length</td>
<td>Raphidosomes not observed</td>
</tr>
<tr>
<td>Helical</td>
<td>Sheathed</td>
</tr>
</tbody>
</table>

Pigmentation

| Type I—red (flexixanthin) | Type IV—yellow (zeaxanthin) |
| Type II—pink             | Type V—yellow               |
| Type III—orange (saproxanthin) | Type VI—red                |

Digestive enzymes

| Liquefies carboxymethylcellulose | Does not liquefy gelatin |
| Does not liquefy carboxymethylcellulose | Liquefies agar |
| Hydrolyses starch                | Does not liquefy agar     |
| Does not hydrolyse starch        | Liquefies alginate        |
| Liquefies gelatin                | Does not liquefy alginate |

Oxidation-reduction enzymes

<table>
<thead>
<tr>
<th>Catalase produced</th>
<th>Catalase not produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S evolved</td>
<td>H₂S not evolved</td>
</tr>
</tbody>
</table>

Reactions to tyrosine (5 g./l.) and dihydroxyphenylalanine (0.1 g./l.)

<table>
<thead>
<tr>
<th>No growth on tyrosine</th>
<th>No growth on dihydroxyphenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth, plus colour change</td>
<td>Growth, plus colour change</td>
</tr>
<tr>
<td>Growth, no colour change</td>
<td>Growth, no colour change</td>
</tr>
<tr>
<td>Growth, plus dissolution</td>
<td>Growth, plus dissolution</td>
</tr>
<tr>
<td>Growth, no dissolution</td>
<td>Growth, no dissolution</td>
</tr>
</tbody>
</table>

Effect of inhibitors

<table>
<thead>
<tr>
<th>Not inhibited by penicillin (10⁻⁴ g./l.)</th>
<th>Not inhibited by lauryl sulphate (0.1 g./l.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibited by penicillin (10⁻⁶ g./l.)</td>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
</tr>
</tbody>
</table>

Vitamin requirements

<table>
<thead>
<tr>
<th>Thiamine required</th>
<th>Cobalamin not required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine not required</td>
<td>Other vitamins not required</td>
</tr>
</tbody>
</table>

Temperature effects

<table>
<thead>
<tr>
<th>Can grow above 40°</th>
<th>Can grow above 30° but not 35°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can grow above 35° but not 40°</td>
<td></td>
</tr>
</tbody>
</table>

Salinity tolerances

| Can grow in above 2 x sea-water media | Cannot grow in diluted sea-water media |
| Can grow in sea water but not 2 x sea-water media | Can grow in diluted sea water but not freshwater media |
| Can grow in ½ x sea water but not sea-water media | Can grow in freshwater media |
Nitrate alone suffices
Glutamate alone suffices, but not nitrate
Arginine required
Aspartic acid required
Histidine required
Glycine required
Isoleucine required
Leucine required
Lysine required
Methionine required

Phenylalanine or tyrosine required
Threonine required
Tryptophan required
Valine required
Asparagine required
Growth on Casamino acids alone (specific requirements unknown)
Growth on Casamino acids plus yeast nucleic acid hydrolysate
Growth on Tryptone but not on Casamino acids

hydrates was also available, but it was decided that the data were too imprecise to use (cf. Dworkin, 1966) and they were, therefore, omitted. Details of experimental procedures will be found in Lewin & Lounsbery (1969). The strain designations used in this paper are the ones used by those authors.

Eighty-five strains of flexibacteria were used. Some strains had positive scores for as many as 33 characteristics, others for as few as 14. The different numbers are the result of two things: incomplete information about some strains and an attempt to minimize the amount of redundant information used. As an example of the latter, a strain that could use nitrate as the sole nitrogen source was not scored as not requiring any of the 13 amino acids tested and was, therefore, recorded only once in regard to the nitrogen requirements, whereas a strain that required specific amino acids could be recorded up to 13 times. These differences may have had some effect on the groupings but every species had enough positive scores to have had affinity (at 0.575) with any of the others.

The grouping procedure gave 19 groups, varying in size from 1 to 19 members. The two single-member groups were strains that showed no affinities with any other strains. Nine strains were close associates but not members of groups. In some cases, their exclusion may have been due to lack of information about these strains.

The groups and associates and their interrelations are shown in Fig. 1. The interrelations are based on determination of the number of pairs of strains that showed affinity between two groups and comparison of this with the possible number. For example, if one group consisted of four strains and another of five, there would be 20 possible between-group pairs of strains and, of these, any number from 0 to 19 might show affinity at or above the preset level. If 20 between-group pairs had shown affinity, the grouping procedure would have put the two groups together in one. As Fig 1. indicates, the groups can be grouped in 7 separate assemblages: groups 1, 12, 18 and 19 by themselves and the rest in three more or less closely connected assemblages of groups. (These assemblages have been designated FA through FG.)

The individual groups are based on similarity of characteristics of their component members and may represent taxonomic entities at the species level. After the groups were determined, the common characteristics of the members of each group were listed. These are shown in Table 2.

The assemblages, based on appreciable but lesser similarity between the groups, may represent taxa at the generic level. For example, in assemblage FC, group 7 consisted of strains that had guanine + cytosine values between 32.5 and 35.0%, were not helical, were yellow, liquefied gelatin, carboxymethylcellulose, agar and alginate,
Table 2. *Characteristics shared by members of recurrent groups of strains*

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helical</td>
<td>Not helical</td>
<td>Not helical</td>
<td>Greater than 50 μm. in length</td>
<td>Not helical</td>
<td>10 to 50 μm. in length</td>
</tr>
<tr>
<td>Rhapidosomes observed</td>
<td>Pigment type III—orange (saproxyanthin)</td>
<td>Pigment type IV—yellow (zeaxanthin)</td>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td>Other vitamins not required</td>
<td>Does not liquefy carboxymethyl cellulose</td>
</tr>
<tr>
<td>Does not hydrolyse starch</td>
<td>Liquefies gelatin</td>
<td>Does not hydrolyse starch</td>
<td>Thiamine required</td>
<td>Isoleucine required</td>
<td>Liquefies gelatin</td>
</tr>
<tr>
<td>Liquefies agar</td>
<td>Does not liquefy carboxymethylcellulose</td>
<td>Does not liquefy agar</td>
<td>Leucine required</td>
<td>Leucine required</td>
<td>Does not liquefy agar</td>
</tr>
<tr>
<td>Catalase not produced</td>
<td>Hydrolyses starch</td>
<td>Does not liquefy alginate</td>
<td>Methionine required</td>
<td>Methionine required</td>
<td>Does not liquefy alginate</td>
</tr>
<tr>
<td>H₂S not evolved</td>
<td>Liquefies alginate</td>
<td>Catalase not produced</td>
<td>Phenylalanine or tyrosine required</td>
<td>Phenylalanine or tyrosine required</td>
<td>Pigment type III—orange (saproxyanthin)</td>
</tr>
<tr>
<td>Growth, no dissolution of tyrosine (5 g./l.)</td>
<td>Catalase not produced</td>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td>Growth, no dissolution of tyrosine (5 g./l.)</td>
<td>Valine required</td>
<td>Does not liquefy carboxymethyl cellulose</td>
</tr>
<tr>
<td>No growth on dihydroxyphenylalanine (0.1 g./l.)</td>
<td>Hydrolyses starch</td>
<td>Growth, no colour change on dihydroxyphenylalanine (0.1 g./l.)</td>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td>Growth, no dissolution of tyrosine (5 g./l.)</td>
<td>Pigment type III—orange (saproxyanthin)</td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td>Liquefies alginate</td>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td>Growth, no dissolution of dihydroxyphenylalanine (0.1 g./l.)</td>
<td>Growth, no dissolution of dihydroxyphenylalanine (0.1 g./l.)</td>
<td>Does not liquefy agar</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Growth, no dissolution of tyrosine (5 g./l.)</td>
<td>Growth, no dissolution of dihydroxyphenylalanine (0.1 g./l.)</td>
<td>Does not liquefy alginate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Can grow in above 2 × sea-water media</td>
<td>Can grow in above 2 × sea-water media</td>
<td></td>
</tr>
</tbody>
</table>
### Flexibacteria: recurrent group analysis

#### Table 2 (cont.)

<table>
<thead>
<tr>
<th>Group 6 cont.</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Catalase not produced</td>
<td>Cobalamin not required</td>
</tr>
<tr>
<td>H₂S evolved</td>
<td>Can grow in freshwater media</td>
</tr>
<tr>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td>Isoleucine required</td>
</tr>
<tr>
<td>Growth, no dissolution of tyrosine (5 g./l.)</td>
<td>Leucine required</td>
</tr>
<tr>
<td>No growth on dihydroxyphenylalanine (0.1 g./l.)</td>
<td>Methionine required</td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td>Valine required</td>
</tr>
<tr>
<td>Guanine + cytosine mole % in DNA, 32.5 to 35.0</td>
<td></td>
</tr>
<tr>
<td>Not helical</td>
<td></td>
</tr>
<tr>
<td>Pigment type IV—yellow (zeaxanthin)</td>
<td></td>
</tr>
<tr>
<td>Liquefies carboxymethylcellulose</td>
<td></td>
</tr>
<tr>
<td>Hydrolyses starch</td>
<td></td>
</tr>
<tr>
<td>Liquefies agar</td>
<td></td>
</tr>
<tr>
<td>Liquefies alginate</td>
<td></td>
</tr>
<tr>
<td>Catalase produced</td>
<td></td>
</tr>
<tr>
<td>Growth, plus colour change on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Thiamine not required</td>
<td></td>
</tr>
<tr>
<td>Cobalamin not required</td>
<td></td>
</tr>
<tr>
<td>Other vitamins not required</td>
<td></td>
</tr>
<tr>
<td>Can grow in above 2 × sea-water media</td>
<td></td>
</tr>
<tr>
<td>Can grow in diluted sea water but not freshwater media</td>
<td></td>
</tr>
<tr>
<td>Glutamate, but not nitrate, suffices as nitrogen source</td>
<td></td>
</tr>
<tr>
<td>10 to 50 μm. in length</td>
<td></td>
</tr>
<tr>
<td>Not helical</td>
<td></td>
</tr>
<tr>
<td>Rhapidosomes not observed</td>
<td></td>
</tr>
<tr>
<td>Liquefies carboxymethylcellulose</td>
<td></td>
</tr>
<tr>
<td>Liquefies gelatin</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy agar</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy alginate</td>
<td></td>
</tr>
<tr>
<td>Catalase not produced</td>
<td></td>
</tr>
<tr>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Greater than 50 μm. in length</td>
<td></td>
</tr>
<tr>
<td>Helical</td>
<td></td>
</tr>
<tr>
<td>Rhapidosomes not observed</td>
<td></td>
</tr>
<tr>
<td>Pigment type V—yellow</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy carboxymethylcellulose</td>
<td></td>
</tr>
<tr>
<td>Hydrolyses starch</td>
<td></td>
</tr>
<tr>
<td>Liquefies gelatin</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy agar</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy alginate</td>
<td></td>
</tr>
<tr>
<td>Catalase not produced</td>
<td></td>
</tr>
<tr>
<td>H₂S evolved</td>
<td></td>
</tr>
<tr>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Guanine + cytosine mole % in DNA, 30.0 to 32.5</td>
<td></td>
</tr>
<tr>
<td>10 to 50 μm. in length</td>
<td></td>
</tr>
<tr>
<td>Not helical</td>
<td></td>
</tr>
<tr>
<td>Rhapidosomes not observed</td>
<td></td>
</tr>
<tr>
<td>Pigment type V—yellow</td>
<td></td>
</tr>
<tr>
<td>Liquefies carboxymethylcellulose</td>
<td></td>
</tr>
<tr>
<td>Liquefies gelatin</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy agar</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy alginate</td>
<td></td>
</tr>
<tr>
<td>Catalase produced</td>
<td></td>
</tr>
<tr>
<td>H₂S not evolved</td>
<td></td>
</tr>
<tr>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td></td>
</tr>
</tbody>
</table>

### Group 7

|  |
|--------------------------|---|
| Growth, plus dissolution of tyrosine (5 g./l.) | |
| Inhibited by lauryl sulphate (0.1 g./l.) | |
| Thiamine not required | |
| Cobalamin not required | |
| Other vitamins not required | |
| Can grow in above 2 × sea-water media | |
| Can grow in diluted sea water but not freshwater media | |
| Glutamate, but not nitrate, suffices as nitrogen source | |

### Group 8

|  |
|--------------------------|---|
| Inhibited by lauryl sulphate (0.1 g./l.) | |
| Thiamine not required | |
| Cobalamin not required | |
| Can grow above 35°C but not 40°C | |
| Can grow in ½ × sea water but not sea-water media | |
| Can grow in freshwater media | |
| Nitrate alone suffices as nitrogen source | |

### Group 9

|  |
|--------------------------|---|
| Growth, no dissolution of tyrosine (5 g./l.) | |
| Inhibited by lauryl sulphate (0.1 g./l.) | |
| Cobalamin required | |
| Can grow above 40°C | |
| Can grow in ½ × sea water but not sea-water media | |
| Can grow in freshwater media | |
| Aspartic acid required | |
| Isoleucine required | |
| Leucine required | |
| Phenylalanine or tyrosine required | |
| Valine required | |

### Group 10

|  |
|--------------------------|---|
| Growth, plus dissolution of tyrosine (5 g./l.) | |
| Growth, no colour change on dihydroxyphenylalanine (0.1 g./l.) | |
| Growth, no dissolution on dihydroxyphenylalanine (0.1 g./l.) | |
| Not inhibited by penicillin (10⁻⁶ g./l.) | |
| Not inhibited by lauryl sulphate (0.1 g./l.) | |
| Thiamine not required | |
| Cobalamin not required | |
| Other vitamins not required | |
| Can grow above 30°C but not 35°C | |
| Can grow in freshwater media | |
| Nitrate alone suffices as nitrogen source | |
Table 2 (cont.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Greater than 50 µm. in length&lt;br&gt;Not helical&lt;br&gt;Sheathed&lt;br&gt;Pigment type III—orange (saproxyanthin)&lt;br&gt;Does not liquefy carboxymethylcellulose&lt;br&gt;Does not hydrolyse starch&lt;br&gt;Does not liquefy agar&lt;br&gt;Does not liquefy alginate&lt;br&gt;Catalase not produced&lt;br&gt;H₂S not produced&lt;br&gt;Growth, no colour change on tyrosine (5 g./l.)&lt;br&gt;Growth, no dissolution on tyrosine (5 g./l.)&lt;br&gt;Growth, no colour change on dihydroxyphenylalanine (0·1 g./l.)&lt;br&gt;Growth, no dissolution on dihydroxyphenylalanine (0·1 g./l.)&lt;br&gt;Inhibited by lauryl sulphate (0·1 g./l.)&lt;br&gt;Can grow above 30° but not 35°&lt;br&gt;Can grow in above 2 x sea-water media&lt;br&gt;Can grow in diluted sea water but not fresh-water media</td>
</tr>
<tr>
<td>12</td>
<td>Guanine + cytosine mole % in DNA, 37·6 to 39·0&lt;br&gt;Not helical&lt;br&gt;Pigment type III—orange (saproxyanthin)&lt;br&gt;Does not liquefy carboxymethylcellulose&lt;br&gt;Hydrolyses starch&lt;br&gt;Does not liquefy gelatin&lt;br&gt;Does not liquefy agar&lt;br&gt;Liquefies alginate&lt;br&gt;Catalase not produced&lt;br&gt;H₂S not produced&lt;br&gt;Growth, no colour change on dihydroxyphenylalanine (0·1 g./l.)&lt;br&gt;Growth, no dissolution on dihydroxyphenylalanine (0·1 g./l.)&lt;br&gt;Inhibited by lauryl sulphate (0·1 g./l.)&lt;br&gt;Can grow above 30° but not 35°&lt;br&gt;Can grow in above 2 x sea-water media&lt;br&gt;Can grow in diluted sea water but not fresh-water media&lt;br&gt;Growth on Tryptone but not on Casamino acids</td>
</tr>
<tr>
<td>13</td>
<td>Guanine + cytosine mole % in DNA, 35·1 to 37·5&lt;br&gt;10 to 50 µm. in length&lt;br&gt;Not helical&lt;br&gt;Pigment type III—orange (saproxyanthin)&lt;br&gt;Does not liquefy carboxymethylcellulose&lt;br&gt;Hydrolyses starch&lt;br&gt;Liquefies gelatin&lt;br&gt;Does not liquefy agar&lt;br&gt;Catalase not produced&lt;br&gt;H₂S evolved&lt;br&gt;Growth, no colour change on tyrosine (5 g./l.)&lt;br&gt;Growth, no dissolution on tyrosine (5 g./l.)&lt;br&gt;Inhibited by lauryl sulphate (0·1 g./l.)&lt;br&gt;Can grow in above 2 x sea-water media&lt;br&gt;Can grow in diluted sea water but not fresh-water media</td>
</tr>
<tr>
<td>14</td>
<td>Guanine + cytosine mole % in DNA 40·1 to 42·5&lt;br&gt;Not helical&lt;br&gt;Pigment type III—orange (saproxyanthin)&lt;br&gt;Liquefies carboxymethylcellulose&lt;br&gt;Hydrolyses starch&lt;br&gt;Liquefies gelatin&lt;br&gt;Liquefies agar&lt;br&gt;Liquefies alginate&lt;br&gt;Catalase not produced&lt;br&gt;H₂S not evolved&lt;br&gt;Growth, no colour change on tyrosine (5 g./l.)&lt;br&gt;Growth, no dissolution on tyrosine (5 g./l.)&lt;br&gt;Not inhibited by penicillin (10⁻⁴ g./l.)&lt;br&gt;Inhibited by lauryl sulphate (0·1 g./l.)&lt;br&gt;Can grow above 30° but not 35°&lt;br&gt;Can grow in above 2 x sea-water media&lt;br&gt;Can grow in diluted sea water but not fresh-water media&lt;br&gt;Can grow in diluted sea water but not fresh-water media&lt;br&gt;Glutamate, but not nitrate suffices as nitrogen source</td>
</tr>
<tr>
<td>15</td>
<td>10 to 50 µm. in length&lt;br&gt;Not helical&lt;br&gt;Rhizoides observed&lt;br&gt;Liquefies carboxymethylcellulose&lt;br&gt;Hydrolyses starch&lt;br&gt;Liquefies gelatin&lt;br&gt;Liquefies agar&lt;br&gt;Liquefies alginate&lt;br&gt;H₂S evolved&lt;br&gt;Growth, no dissolution on tyrosine (5 g./l.)&lt;br&gt;No growth on dihydroxyphenylalanine (0·1 g./l.)&lt;br&gt;Not inhibited by penicillin (10⁻⁴ g./l.)&lt;br&gt;Thiamine not required&lt;br&gt;Cobalamin not required&lt;br&gt;Other vitamins not required&lt;br&gt;Can grow above 35° but not 40°&lt;br&gt;Can grow in above 2 x sea-water media&lt;br&gt;Can grow in diluted sea water but not fresh-water media&lt;br&gt;Glutamate, but not nitrate suffices as nitrogen source</td>
</tr>
</tbody>
</table>
### Table 2 (cont.)

#### Group 16

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanine + cytosine mole % in DNA</td>
<td>32.6 to 35.0</td>
</tr>
<tr>
<td>Less than 10 µm. in length</td>
<td></td>
</tr>
<tr>
<td>Not helical</td>
<td></td>
</tr>
<tr>
<td>Rhapidosomes not observed</td>
<td></td>
</tr>
<tr>
<td>Pigment type IV—yellow (zeaxanthin)</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy carboxymethylcellulose</td>
<td></td>
</tr>
<tr>
<td>Liquefies gelatin</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy agar</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy alginate</td>
<td></td>
</tr>
<tr>
<td>Catalase not produced</td>
<td></td>
</tr>
<tr>
<td>H₂S not evolved</td>
<td></td>
</tr>
<tr>
<td>Growth, plus dissolution on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>No growth on dihydroxyphenylalanine (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Thiamine not required</td>
<td></td>
</tr>
<tr>
<td>Cobalamin not required</td>
<td></td>
</tr>
<tr>
<td>Other vitamins not required</td>
<td></td>
</tr>
<tr>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Growth, no dissolution on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Can grow in freshwater media</td>
<td></td>
</tr>
</tbody>
</table>

#### Group 17

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanine + cytosine mole % in DNA</td>
<td>40.1 to 42.5</td>
</tr>
<tr>
<td>Greater than 50 µm. in length</td>
<td></td>
</tr>
<tr>
<td>Not helical</td>
<td></td>
</tr>
<tr>
<td>Rhapidosomes observed</td>
<td></td>
</tr>
<tr>
<td>Pigment type III—orange (saproxanthin)</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy carboxymethylcellulose</td>
<td></td>
</tr>
<tr>
<td>Liquefies gelatin</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy agar</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy alginate</td>
<td></td>
</tr>
<tr>
<td>Catalase not produced</td>
<td></td>
</tr>
<tr>
<td>H₂S not evolved</td>
<td></td>
</tr>
<tr>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Growth, no dissolution on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Can grow in freshwater media</td>
<td></td>
</tr>
</tbody>
</table>

#### Group 18

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanine + cytosine mole % in DNA</td>
<td>42.5 to 45.0</td>
</tr>
<tr>
<td>Greater than 50 µm. in length</td>
<td></td>
</tr>
<tr>
<td>Not helical</td>
<td></td>
</tr>
<tr>
<td>Rhapidosomes observed</td>
<td></td>
</tr>
<tr>
<td>Pigment type III—orange (saproxanthin)</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy carboxymethylcellulose</td>
<td></td>
</tr>
<tr>
<td>Liquefies gelatin</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy agar</td>
<td></td>
</tr>
<tr>
<td>Does not liquefy alginate</td>
<td></td>
</tr>
<tr>
<td>Catalase not produced</td>
<td></td>
</tr>
<tr>
<td>H₂S not evolved</td>
<td></td>
</tr>
<tr>
<td>Growth, no colour change on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Growth, plus dissolution on tyrosine (5 g./l.)</td>
<td></td>
</tr>
<tr>
<td>No growth on dihydroxyphenylalanine (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Not inhibited by penicillin (10⁻⁶ g./l.)</td>
<td></td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Can grow above 30°C but not 35°C</td>
<td></td>
</tr>
<tr>
<td>Can grow in above 2 × sea-water media</td>
<td></td>
</tr>
<tr>
<td>Cannot grow in diluted sea-water media</td>
<td></td>
</tr>
<tr>
<td>Growth on Tryptone but not on Casamino acids</td>
<td></td>
</tr>
</tbody>
</table>

#### Group 19

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanine + cytosine percentage in DNA</td>
<td>42.6 to 45.0</td>
</tr>
<tr>
<td>Greater than 50 µm. in length</td>
<td></td>
</tr>
<tr>
<td>Not helical</td>
<td></td>
</tr>
<tr>
<td>Rhapidosomes observed</td>
<td></td>
</tr>
<tr>
<td>Sheathed</td>
<td></td>
</tr>
<tr>
<td>Pigment type III—orange (saproxanthin)</td>
<td></td>
</tr>
<tr>
<td>Liquefies agar</td>
<td></td>
</tr>
<tr>
<td>Liquefies alginate</td>
<td></td>
</tr>
<tr>
<td>Inhibited by lauryl sulphate (0.1 g./l.)</td>
<td></td>
</tr>
<tr>
<td>Thiamine not required</td>
<td></td>
</tr>
<tr>
<td>Cobalamin not required</td>
<td></td>
</tr>
<tr>
<td>Other vitamins not required</td>
<td></td>
</tr>
<tr>
<td>Glutamate, but not nitrate, suffices as nitrogen source</td>
<td></td>
</tr>
</tbody>
</table>

hydrolysed starch, grew on tyrosine medium with colour change and dissolution, could use glutamate but not nitrate as nitrogen source, and grew in 2 × to 3 × sea-water media. Group 15 was made up of strains with much the same characteristics except that it contained both orange and yellow strains, had guanine + cytosine values between 32.5 and 37.5%, and growth on tyrosine medium did not result in dissolution and was variable in colour change. The strains in group 14 were similar to those in group 15 but had guanine + cytosine values between 40 and 42.5%, were both orange, and did not produce a colour change on tyrosine medium; no information was available on their nitrogen requirements. Group 2 consisted of strains much like those in group 14 except that they had guanine + cytosine values ranging from 30 to
Fig. 1. Groups and interrelations of groups among 85 strains of flexibacteria, as defined by a computer programme for determination of recurrent groups. The groups are numbered 1 through 19. Associates are shown in the small boxes connected to the groups. Assemblages of groups are designated FA through FG. Heavier lines = $\geq 4$ or more of the possible connections between groups realized; lighter lines = between $\frac{4}{3}$ and $\frac{1}{3}$ of the possible connections realized (see text).

45%, and one strain (HI 1) which did not liquefy agar or gelatin. The groups in this assemblage (FC) have, therefore, considerable similarity.

The arrangement of the strains in groups and of the groups in assemblages is based on overall similarities using all of the information available. All of the characters were given equal weight. As Sneath (1962) pointed out, this procedure leads to a consistent taxonomy, and is particularly useful when the aim is to form taxa of greatest information content. Although the groups and assemblages suggested by the computer analysis have been used for the most part by Lewin (1969) as a basis for classification, he has in some cases departed from them because he felt that certain characteristics should be given more weight than others in defining species and genera. A detailed discussion of his reasons for the changes is given in his paper.
REFERENCES


