Recurrent Group Analysis in the Classification of Flexibacteria

By E. W. FAGER

*Scripps Institution of Oceanography, University of California, La Jolla,
California 92037, U.S.A.*

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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 ($J/\sqrt{(A \times B)} - 1/2\sqrt{B}$; 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-

* FORTRAN listings are available for the grouping programme (REGROUP), the programme for determining connections between groups (CONNEX), and the programme for listing the characteristics shared by group members (STATION). These have been programmed for the CDC 3600.

Table 1. *Characteristics recorded, based on the data of Lewin & Lounsberg (1969).*

Guanine + cytosine mole % in DNA	
Less than 30 %	40.1 to 42.5
30.0 to 32.5	42.6 to 45.0
32.6 to 35.0	45.1 to 47.5
35.1 to 37.5	47.6 to 50.0
37.6 to 40.0	Greater than 50.0
Morphology, etc.	
Less than 10 μ in length	Not helical
10 to 50 μ in length	Rhaphidosomes observed
Greater than 50 μ in length	Rhaphidosomes not observed
Helical	Sheathed
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	
Catalase produced	H ₂ S evolved
Catalase not produced	H ₂ S not evolved
Reactions to tyrosine (5 g./l.) and dihydroxyphenylalanine (0.1 g./l.)	
No growth on tyrosine	No growth on dihydroxyphenylalanine
Growth, plus colour change	Growth, plus colour change
Growth, no colour change	Growth, no colour change
Growth, plus dissolution	Growth, plus dissolution
Growth, no dissolution	Growth, no dissolution
Effect of inhibitors	
Not inhibited by penicillin (10 ⁻⁶ g./l.)	Not inhibited by lauryl sulphate (0.1 g./l.)
Inhibited by penicillin (10 ⁻⁶ g./l.)	Inhibited by lauryl sulphate (0.1 g./l.)
Vitamin requirements	
Thiamine required	Cobalamin not required
Thiamine not required	Other vitamins not required
Cobalamin required	
Temperature effects	
Can grow above 40°	Can grow above 30° but not 35°
Can grow above 35° but not 40°	
Salinity tolerances	
Can grow in above 2 × sea-water media	Cannot grow in diluted sea-water media
Can grow in sea water but not 2 × sea-water media	Can grow in diluted sea water but not fresh-water media
Can grow in $\frac{1}{2}$ × sea water but not sea-water media	Can grow in freshwater media

Table 1 (*cont.*)

Nitrogen requirements

Nitrate alone suffices	Phenylalanine or tyrosine required
Glutamate alone suffices, but not nitrate	Threonine required
Arginine required	Tryptophan required
Aspartic acid required	Valine required
Histidine required	Asparagine required
Glycine required	Growth on Casamino acids alone (specific requirements unknown)
Isoleucine required	Growth on Casamino acids plus yeast nucleic acid hydrolysate
Leucine required	Growth on Tryptone but not on Casamino acids
Lysine required	
Methionine required	

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 & Lounsbury (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 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*

Group 1	
Helical	Arginine required
Rhaphidosomes observed	Aspartic acid required
Pigment type III—orange (saproxanthin)	Histidine required
Does not hydrolyse starch	Iso-leucine required
Liquefies gelatin	Leucine required
Does not liquefy agar	Lysine required
Catalase not produced	Methionine required
H ₂ S not evolved	Phenylalanine or tyrosine required
Growth, no dissolution of tyrosine (5 g./l.)	Threonine required
No growth on dihydroxyphenylalanine (0.1 g./l.)	Tryptophan required
Inhibited by lauryl sulphate (0.1 g./l.)	Valine required
	Asparagine required
Group 2	
Not helical	H ₂ S evolved
Pigment type III—orange (saproxanthin)	Growth, no colour change on tyrosine (5 g./l.)
Liquefies carboxymethylcellulose	Growth, no dissolution of tyrosine (5 g./l.)
Hydrolyses starch	Can grow in above 2 × sea-water media
Liquefies alginate	Can grow in diluted sea water but not fresh-water media
Catalase not produced	
Group 3	
Not helical	Growth, no dissolution of tyrosine (5 g./l.)
Pigment type IV—yellow (zeaxanthin)	Growth, no colour change on dihydroxyphenylalanine (0.1 g./l.)
Does not hydrolyse starch	Inhibited by lauryl sulphate (0.1 g./l.)
Does not liquefy agar	Can grow in above 2 × sea-water media
Does not liquefy alginate	Can grow in diluted sea water but not fresh-water media
Catalase not produced	
H ₂ S not evolved	
Growth, no colour change on tyrosine (5 g./l.)	
Group 4	
Greater than 50 μm. in length	Inhibited by lauryl sulphate (0.1 g./l.)
Not helical	Thiamine required
Rhaphidosomes not observed	Other vitamins not required
Does not liquefy carboxymethylcellulose	Can grow in freshwater media
Liquefies gelatin	Isoleucine required
Does not liquefy agar	Leucine required
Does not liquefy alginate	Methionine required
Catalase not produced	Phenylalanine or tyrosine required
No growth on tyrosine (5 g./l.)	Valine required
No growth on dihydroxyphenylalanine (0.1 g./l.)	
Group 5	
Not helical	Growth, no dissolution of tyrosine (5 g./l.)
Does not liquefy agar	Growth, no dissolution of dihydroxyphenylalanine (0.1 g./l.)
Does not liquefy alginate	Inhibited by lauryl sulphate (0.1 g./l.)
Catalase not produced	Can grow in above 2 × sea-water media
Growth, no colour change on tyrosine (5 g./l.)	
Group 6	
10 to 50 μm. in length	Does not liquefy carboxymethyl cellulose
Not helical	Liquefies gelatin
Rhaphidosomes not observed	Does not liquefy agar
Pigment type III—orange (saproxanthin)	Does not liquefy alginate

Table 2 (*cont.*)Group 6 *cont.*

Catalase not produced	Cobalamin not required
H ₂ S evolved	Can grow in freshwater media
Growth, no colour change on tyrosine (5 g./l.)	Isoleucine required
Growth, no dissolution of tyrosine (5 g./l.)	Leucine required
No growth on dihydroxyphenylalanine (0.1 g./l.)	Methionine required
Inhibited by lauryl sulphate (0.1 g./l.)	Valine required

Group 7

Guanine + cytosine mole % in DNA, 32.5 to 35.0	Growth, plus dissolution of tyrosine (5 g./l.)
Not helical	Inhibited by lauryl sulphate (0.1 g./l.)
Pigment type IV—yellow (zeaxanthin)	Thiamine not required
Liquefies carboxymethylcellulose	Cobalamin not required
Hydrolyses starch	Other vitamins not required
Liquefies agar	Can grow in above 2 × sea-water media
Liquefies alginate	Can grow in diluted sea water but not fresh-water media
Catalase produced	Glutamate, but not nitrate, suffices as nitrogen source
Growth, plus colour change on tyrosine (5 g./l.)	

Group 8

10 to 50 μm. in length	Inhibited by lauryl sulphate (0.1 g./l.)
Not helical	Thiamine not required
Rhaphidosomes not observed	Cobalamin not required
Liquefies carboxymethylcellulose	Can grow above 35° but not 40°
Liquefies gelatin	Can grow in ½ × sea water but not sea-water media
Does not liquefy agar	Can grow in freshwater media
Does not liquefy alginate	Nitrate alone suffices as nitrogen source
Catalase not produced	

Group 9

Greater than 50 μm. in length	Growth, no dissolution of tyrosine (5 g./l.)
Helical	Inhibited by lauryl sulphate (0.1 g./l.)
Rhaphidosomes not observed	Cobalamin required
Pigment type II—pink	Can grow above 40°
Does not liquefy carboxymethylcellulose	Can grow in ½ × sea water but not sea-water media
Hydrolyses starch	Can grow in freshwater media
Liquefies gelatin	Aspartic acid required
Does not liquefy agar	Isoleucine required
Does not liquefy alginate	Leucine required
Catalase not produced	Phenylalanine or tyrosine required
H ₂ S evolved	Valine required
Growth, no colour change on tyrosine (5 g./l.)	

Group 10

Guanine + cytosine mole % in DNA, 30.0 to 32.5	Growth, plus dissolution of tyrosine (5 g./l.)
10 to 50 μm. in length	Growth, no colour change on dihydroxy-phenylalanine (0.1 g./l.)
Not helical	Growth, no dissolution on dihydroxyphenylalanine (0.1 g./l.)
Rhaphidosomes not observed	Not inhibited by penicillin (10 ⁻⁶ g./l.)
Pigment type V—yellow	Not inhibited by lauryl sulphate (0.1 g./l.)
Liquefies carboxymethylcellulose	Thiamine not required
Liquefies gelatin	Cobalamin not required
Does not liquefy agar	Other vitamins not required
Does not liquefy alginate	Can grow above 30° but not 35°
Catalase produced	Can grow in freshwater media
H ₂ S not evolved	Nitrate alone suffices as nitrogen source
Growth, no colour change on tyrosine (5 g./l.)	

Table 2 (*cont.*)

Group 11	
Greater than 50 μ m. in length	Growth, no dissolution on tyrosine (5 g./l.)
Not helical	Growth, no colour change on dihydroxy-phenylalanine (0.1 g./l.)
Sheathed	Growth, no dissolution on dihydroxyphenylalanine (0.1 g./l.)
Pigment type III—orange (saproxanthin)	Inhibited by lauryl sulphate (0.1 g./l.)
Does not liquefy carboxymethylcellulose	Can grow above 30° but not 35°
Does not hydrolyse starch	Can grow in above 2 $\times$ sea-water media
Does not liquefy agar	Can grow in diluted sea water but not fresh-water media
Does not liquefy alginate	
Catalase not produced	
H ₂ S not evolved	
Growth, no colour change on tyrosine (5 g./l.)	
Group 12	
Guanine + cytosine mole % in DNA, 37.6 to 40.0	Growth, no colour change on dihydroxy-phenylalanine (0.1 g./l.)
Not helical	Growth, no dissolution on dihydroxyphenylalanine (0.1 g./l.)
Pigment type III—orange (saproxanthin)	Inhibited by lauryl sulphate (0.1 g./l.)
Does not liquefy carboxymethylcellulose	Can grow above 30° but not 35°
Hydrolyses starch	Can grow in above 2 $\times$ sea-water media
Does not liquefy gelatin	Can grow in diluted sea water but not fresh-water media
Does not liquefy agar	Growth on Tryptone but not on Casamino acids
Liquefies alginate	
Catalase not produced	
H ₂ S not evolved	
Group 13	
Guanine + cytosine mole % in DNA, 35.1 to 37.5	Does not liquefy agar
10 to 50 μ m. in length	Does not liquefy alginate
Not helical	Catalase not produced
Pigment type III—orange (saproxanthin)	H ₂ S evolved
Does not liquefy carboxymethylcellulose	Growth, no colour change on tyrosine (5 g./l.)
Hydrolyses starch	Growth, no dissolution on tyrosine (5 g./l.)
Liquefies gelatin	Inhibited by lauryl sulphate (0.1 g./l.)
	Can grow in above 2 $\times$ sea-water media
Group 14	
Guanine + cytosine mole % in DNA 40.1 to 42.5	H ₂ S not evolved
Not helical	Growth, no colour change on tyrosine (5 g./l.)
Pigment type III—orange (saproxanthin)	Growth, no dissolution on tyrosine (5 g./l.)
Liquefies carboxymethylcellulose	Not inhibited by penicillin (10 ⁻⁶ g./l.)
Hydrolyses starch	Inhibited by lauryl sulphate (0.1 g./l.)
Liquefies gelatin	Can grow above 30° but not 35°
Liquefies agar	Can grow in above 2 $\times$ sea-water media
Liquefies alginate	Can grow in diluted sea water but not fresh-water media
Catalase not produced	
Group 15	
10 to 50 μ m. in length	Not inhibited by penicillin (10 ⁻⁶ g./l.)
Not helical	Thiamine not required
Rhaphidosomes observed	Cobalamin not required
Liquefies carboxymethylcellulose	Other vitamins not required
Hydrolyses starch	Can grow above 35° but not 40°
Liquefies gelatin	Can grow in above 2 $\times$ sea-water media
Liquefies agar	Can grow in diluted sea water but not fresh-water media
Liquefies alginate	Glutamate, but not nitrate suffices as nitrogen source
H ₂ S evolved	
Growth, no dissolution on tyrosine (5 g./l.)	
No growth on dihydroxyphenylalanine (0.1 g./l.)	

Table 2 (cont.)

Group 16	
Guanine + cytosine mole % in DNA, 32.6 to 35.0	Does not liquefy alginate
Less than 10 μm . in length	Catalase not produced
Not helical	H ₂ S not evolved
Rhaphidosomes not observed	Growth, plus dissolution on tyrosine (5 g./l.)
Pigment type IV—yellow (zeaxanthin)	No growth on dihydroxyphenylalanine (0.1 g./l.)
Does not liquefy carboxymethylcellulose	Inhibited by lauryl sulphate (0.1 g./l.)
Liquefies gelatin	Thiamine not required
Does not liquefy agar	Cobalamin not required
	Other vitamins not required
Group 17	
Not helical	Does not liquefy alginate
Rhaphidosomes not observed	Catalase not produced
Pigment type III—orange (saproxanthin)	H ₂ S not evolved
Does not liquefy carboxymethylcellulose	Growth, no colour change on tyrosine (5 g./l.)
Does not hydrolyse starch	Growth, no dissolution on tyrosine (5 g./l.)
Liquefies gelatin	Inhibited by lauryl sulphate (0.1 g./l.)
Does not liquefy agar	Can grow in freshwater media
Group 18	
Guanine + cytosine mole % in DNA, 40.1 to 42.5	Growth, no colour change on tyrosine (5 g./l.)
Greater than 50 μm . in length	Growth, plus dissolution on tyrosine (5 g./l.)
Not helical	No growth on dihydroxyphenylalanine (0.1 g./l.)
Rhaphidosomes observed	Not inhibited by penicillin (10 ⁻⁶ g./l.)
Pigment type III—orange (saproxanthin)	Inhibited by lauryl sulphate (0.1 g./l.)
Does not liquefy carboxymethylcellulose	Can grow above 30° but not 35°
Liquefies gelatin	Can grow in above 2 $\times$ sea-water media
Does not liquefy agar	Cannot grow in diluted sea-water media
Does not liquefy alginate	Growth on Tryptone but not on Casamino acids
Catalase not produced	
H ₂ S not evolved	
Group 19	
Guanine + cytosine percentage in DNA, 42.6 to 45.0	Liquefies alginate
10 to 50 μm . in length	Inhibited by lauryl sulphate (0.1 g./l.)
Not helical	Thiamine not required
Rhaphidosomes observed	Cobalamin not required
Sheathed	Other vitamins not required
Pigment type III—orange (saproxanthin)	Glutamate, but not nitrate, suffices as nitrogen source
Liquefies agar	

hydrolysed starch, grew on tyrosine medium with colour change and dissolution, could use glutamate but not nitrate as nitrogen source, and grew in $2 \times$ to $\frac{1}{2} \times$ 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 = $\frac{1}{2}$ or more of the possible connections between groups realized; lighter lines = between $\frac{1}{4}$ and $\frac{1}{2}$ of the possible connections realized (see text).

45%, and one strain (HJ 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.

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