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Approach to an Improved Taxonomy of the genus *Agrobacterium*

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SUMMARY

With a view to an improved taxonomy of the genus *Agrobacterium*, 45 strains, including representatives of all nomen-species, were used to investigate the following features: base composition and compositional distribution of pure deoxyribonucleic acid (DNA); type of flagellation; 3-ketoglycoside formation; phytopathogenicity for tomato and Datura. It is proposed to limit the genus *Agrobacterium* to two, or possibly three, species: (1) *Agrobacterium radiobacter* and its phytopathogenic variety *A. radiobacter* var. *tumefaciens*; (2) *A. rhizogenes*; and (3) possibly *A. pseudotsugae*. More work on the latter two species is required before they can definitely be accepted as separate species of this genus. The DNA of all the strains of the former two species has a T_m value in the narrow range of 93·8°–95·1°, corresponding with an average molar guanine + cytosine (GC) content of 59·5–62·8%. The variance σ of the compositional distribution of the DNA molecules ranges between 0 and 0·88% with an average of 0·24% (GC). The only available strain of *A. pseudotsugae*, with 67·7% GC, was completely out of this range and its chromosomal DNA was clearly different from that of the other two species. All strains of *Agrobacterium* proper were peritrichous, frequently with 5–6 flagella. All 8 strains of *A. radiobacter* and 24 of 28 strains of the variety '*tumefaciens*' converted lactose into 3-ketolactose; all the other strains were negative in this respect. Several arguments are advanced to include the strain *A. rubi* with *A. radiobacter* var. *tumefaciens* and to remove the species *A. stellulatum* and *A. gypsophilae* from this genus. The relationship between *Agrobacterium* and some other genera is shown graphically in a plot of mean similarity *versus* DNA base composition.

INTRODUCTION

The present taxonomy of the genus *Agrobacterium* is confused and varies from one textbook to another. Both *Bergey's Manual* (1957) and Prévot (1961) recognize *Agrobacterium* as a separate genus. The former text includes five phytopathogenic species (*A. tumefaciens*, *A. gypsophilae*, *A. pseudotsugae*, *A. rhizogenes*, *A. rubi*) and two non-phytopathogenic ones (*A. radiobacter*, *A. stellulatum*). Prévot (1961) recognizes only *A. tumefaciens*, *A. rhizogenes*, *A. rubi* and *A. pseudotsugae*, and includes *gypsophilae* in *Xanthomonas*. On the other hand, Krassilnikov (1959) does not mention the generic name *Agrobacterium* and includes the species '*tumefaciens*', '*rhizogenes*', '*gypsophilae*' and '*radiobacter*' in *Pseudomonas*; the other nomen-species are not mentioned. Dowson (1957) included the species '*tumefaciens*' and '*rhizogenes*' in *Erwinia*. The genus *Agrobacterium* as defined for example in *Bergey's Manual* (1957) is very heterogeneous and would contain both polarly and peritrichously flagellated organisms. The host-specificity range of some of the species (e.g. *A. rubi*) is poorly known and their classification is partially built on trifles such as small differenees in nitrate reduction. A revision of this genus is thus much needed. Because DNA base composition and flagellation appeared to be useful features for an improved and simplified classification of the genus *Rhizobium* (De Ley & Rassel, 1965) we applied the same approach with a group of 45 agrobacteria, including representatives of each of the seven nomen-species. These organisms have few specific physiological and biochemical characteristics, except their phytopathogenicity and the production of 3-ketoglycosides from disaccharides and bionic acids. These features were also examined in our survey. Phytopathogenicity tests were limited to the use of tomato and Datura and were mainly intended as a control. The formation of 3-ketoglycosides was discovered in our laboratories (Bernaerts & De Ley, 1958; 1960*a*, *b*; 1961; 1963; Bernaerts, Furnelle & De Ley, 1963) and later confirmed elsewhere (Fukui *et al.* 1963; Fukui & Hochster, 1963*a*, *b*).

METHODS

Organisms. All the organisms used are listed in Table 1. Many of the strains were kindly given by the National Collection of Plant Pathogenic Bacteria (NCPPB: Ministry of Agriculture, Fisheries and Food, Hatching Green, Harpenden, England); the International Collection of Phytopathogenic Bacteria (ICPB; Department of Bacteriology, University of California, Davis, Calif., U.S.A.); the National Collection of Industrial Bacteria (NCIB; Torry Research Station, Aberdeen, Scotland); the Institut Pasteur, Paris, France; the Institut Agronomique de l'Etat, Gembloux, Belgium; the Laboratory for Microbiology, Technological University, Delft, the Netherlands. *Agrobacterium stellulatum* was obtained through the courtesy of Professor C. Stapp (Institut für Bakteriologie und Serologie, Braunschein-Gleismarode, Germany). Several strains were bought from the American Type Culture Collection (ATCC; Washington, D.C. U.S.A.). The strains *A. radiobacter* L/2/2/1 and M 2/1 were the ones with which 3-ketoglycoside production was first observed (Bernaerts & De Ley, 1958; 1960*a*, *b*).

Estimation of DNA base composition by thermal denaturation. Mass cultures of the organisms were prepared by growing them for about 2 days at 30° in Roux flasks on a medium containing (%, w/v): glucose, 1; yeast extract (Nederlandse Gist- en

Spiritusfabriek, Brugge, Belgium), 1; agar, 2·5. The bacteria were harvested and pure DNA prepared therefrom according to Marmur (1961). Thermal denaturation was determined with the recording thermospectrophotometer described previously (De Ley & Van Muylem, 1963). The midpoint of the thermal transition T_m and the variance σ expressed as % guanine + cytosine (GC) of the compositional distribution of DNA molecules were determined graphically from the recorded absorbance-temperature curves. The mean % GC was calculated with Marmur & Doty's (1962) formula, % GC = $(T_m - 69{\cdot}3)/0{\cdot}41$. A previous control of this formula with the acetic acid bacteria (De Ley & Schell, 1963) had shown it to be reliable in the present temperature range.

Flagellation. Since the flagella of Agrobacterium are easily detached during the ordinary staining procedure, electron microscopy was used. The methods were the same as in the previous paper (De Ley & Rassel, 1965) except that the suspending medium was much diluted, which decreased the background impurities, and that instead of 2%, 0·5% phosphotungstic acid was used, which produced sharper cell edges. Each strain was observed repeatedly at different growth stages.

Phytopathogenicity. A dense suspension of each bacterial strain was inoculated into the freshly wounded upper stem of two plants each of *Datura stramonium* and *Lycopersicon esculentum.* Tumour formation was recorded after 8–10 days. Tumourigenic strains always acted on both types of plants. No effort was made to test for the hairy-root disease attributed to *Agrobacterium rhizogenes.*

3-Ketoglycoside production was determined by the auxanographic method of Bernaerts & De Ley (1963).

RESULTS AND DISCUSSION

All the results are listed in Table 1.

General properties of Agrobacterium

There seems to be some hope that the knowledge of the % guanine + cytosine (GC) of the bacterial DNAs might partially help to clarify the present definition and classification of the genus *Agrobacterium.* All strains of *Agrobacterium radiobacter*, *A. tumefaciens* and *A. rhizogenes* had a T_m value in the very narrow range of 93·8°–95·1° (total range 1·3°) with a corresponding % GC of 59·5 to 62·8. Taken in conjunction with the other known morphological and physiological properties of these organisms, this indicates a possible very close genetic relationship.

All strains of these three groups are peritrichously flagellated, frequently with 5–6 flagella (Pl. 1). The largest flagella are about 4–5 times as long as the bacterium and have a diameter of 100–120 Å. Frequently 2–10 organisms stick together and single organisms are rare; this makes it sometimes difficult to count the exact number of flagella. One or two of the flagella are often subpolarly implanted. This is strongly reminescent of a similar situation encountered with *Rhizobium* (De Ley & Rassel, 1965) and may be another argument in favour of a close relationship between the two groups. Quite frequently the bacteria bear fimbriae-like structures which are longer, thinner (diameter 60–80 Å), more rigid and more fragile than the flagella.

Concerning the phytopathogenicity, it is seen from Table 1 that there were very few misnomers in *Agrobacterium tumefaciens*, except the strains '*rhizogenes*' NCPPB 5

Table 1. *Genotypic (expressed as 'melting point' T_m, % guanine+cytosine (GC) and variance σ of pure DNA) and phenotypic (tumourigenesis, type of flagellation, 3-ketolactose formation) properties of several strains of Agrobacterium*

For methods, see text. The peritrichous nature of the strains marked* was not as clear-cut as in the other cases.

Species name as received	Origin	Strain number	T_m in °C.	% GC	σ	Tumourigenesis	Flagellation	3-ketolactose formation
		Proposed name: *Agrobacterium radiobacter* var. *tumefaciens*						
tumefaciens	NCPPB	398	93·9	59·9	0·62	+ + +	Peri	+ +
tumefaciens	Gembloux	C	94·1	60·3	0·25	+ +	Peri	+ +
tumefaciens	NCPPB	396	94·2	60·6	0·88	+ + +	Peri	+ + +
tumefaciens	ICPB	TT 111	94·2	60·6	0	+ + + +	Peri	+ +
tumefaciens	ICPB	TT 6	94·2	60·6	0·25	+ + +	Peri	+ +
rhizogenes	NCPPB	5	94·25	60·7	0	+ +	Peri	+ + +
tumefaciens	ATCC	11·158	94·3	60·8	0·31	+ +	Peri	+ +
tumefaciens	ATCC	143	94·35	60·9	0·31	+ +	Peri	+ +
tumefaciens	ATCC	4452	94·35	60·9	0·50	+ +	Peri	+ +
tumefaciens	ATCC	11·156	94·4	61·1	0·25	+ +	Peri	+ +
tumefaciens	Gembloux	A	94·4	61·1	0·12	+ +	Peri	+ +
tumefaciens	ATCC	11·157	94·5	61·3	0·12	+ +	Peri	+ +
tumefaciens	Inst. Pasteur	42 IV	94·5	61·3	0·6	+	Peri	+ + +
tumefaciens	Inst. Pasteur	B 6	94·55	61·4	0	+ + +	Peri*	+ + +
tumefaciens	NCPPB	4	94·55	61·4	0·25	+ + +	Peri	+ + +
tumefaciens	ICPB	TT 9	94·6	61·5	0	+ +	Peri	+ +
tumefaciens	ICPB	TT 107	94·7	61·8	0	+ +	Peri*	+ +
tumefaciens	Gembloux	tum 6	94·6	61·5	0	+ +	Peri	+ +
tumefaciens	NCPPB	925	94·7	61·8	0·19	+ + +	Peri	+ +
tumefaciens	Inst. Pasteur	S 1	94·7	61·8	0·2	+ +	Peri*	+ +
tumefaciens	ATCC	4720	94·8	62·1	0·13	+ +	Peri	+ +
tumefaciens	NCPPB	397	94·85	62·2	0·31	+ + +	Peri	+ + +
tumefaciens	Delft	F	94·9	62·3	0·25	+ +	Peri	+ +
radiobacter	Delft	G	94·9	62·3	0	+ +	Peri	+ +
rubi	ICPB	TR 2	93·8	59·6	0	+ + +	Peri	−
tumefaciens	NCPPB	223	94·7	61·8	0	+ +	Peri	−
tumefaciens	NCPPB	794	94·9	62·3	0·25	+	Peri	−
tumefaciens	ICPB	TT 133	95·0	62·5	0·25	+ + +	Peri	−
		Proposed name: *Agrobacterium radiobacter*						
radiobacter	NCIB	8149	94·15	60·5	0·25	−	Peri	+ +
tumefaciens	Inst. Pasteur	RV 3	94·25	60·7	0·81	−	Peri	+ + +
radiobacter	ICPB	TR 1	84·45	61·2	0·25	−	Peri	+ + +
radiobacter	Gembloux	S 1005	94·5	61·3	0·06	−	Peri	+ +
radiobacter	ATCC	4718	94·7	61·8	0·4	−	Peri	+ +
radiobacter	Own isolate	M 2/1	94·8	62·1	0	−	Peri	+ +
radiobacter	ICPB	TR 6	94·8	62·1	0	−	Peri	+ + +
radiobacter	Own isolate	L 2/2/1	95·0	62·5	0	−	Peri	+ +
		Proposed name: *Agrobacterium rhizogenes*						
rhizogenes	ICPB	TR 101	94·5	61·1	0·37	−	Peri	−
rhizogenes	ICPB	TR 107	94·9	62·3	0	±	Peri	−
rhizogenes	ICPB	TR 7	95·1	62·8	0·37	−	Peri	−
rhizogenes	NCIB	8196	95·1	62·8	0·66	−	Peri	−
		Doubtful cases						
tumefaciens	ATCC	11·095	94·4	61·1	0·62	−	Polar	−
tumefaciens	NCPPB	930	93·5	58·9	0·12	−	Peri	−
pseudotsugae	NCPPB	180	97·1	67·7	0·13	±	Peri	−
peucephilae	NCPPB	179	92·4	56·2	2·5	−	Peri	−
stellulatum	Stapp	2216	96·4	66·0	0·63	−	Polar	−

and '*radiobacter*' G. There appeared to be none in '*radiobacter*'; we propose to relegate the strain RV 3 to '*radiobacter*' because it was not pathogenic.

The formation of 3-ketoglycosides appears to be specific for *Agrobacterium tumefaciens* and *A. radiobacter*; it was originally proposed as a quick and specific test for the crown-gall bacteria (Bernaerts & De Ley, 1963). The present examination of a much larger group of strains showed that 24 of 28 strains of *A. tumefaciens* and all 8 strains of *A. radiobacter* were positive. All other organisms were negative; nor did they produce 3-keto derivatives from maltose or sucrose. The test of Bernaerts & De Ley was used successfully by Béaud (1964) and Manigault & Béaud (1963). The present results further strengthen the practical applicability of this test: when an unknown strain, isolated from a diseased plant supposed to have crown gall, shows a positive test it belongs to the '*tumefaciens-radiobacter*' group; '*radiobacter*' is excluded because it is not pathogenic; the nature of the disease can thus quickly be established. Since 4 of 86 of our strains did not produce 3-ketoglycoside, a negative outcome of the test is no proof that no *A. tumefaciens* is involved; in this case one has to resort to the lengthy phytopathogenic identification. Grebner, Durbin & Feingold (1964) questioned the specificity of the test of Bernaerts & De Ley because a *Micrococcus* sp. produced 3-ketosucrose from sucrose. However, the amounts produced were so small that they were only detected by paper chromatography and the authors reported that the test of Bernaerts & De Ley was negative. Agrobacterium on the other hand converts frequently 75–100 % of the substrate into the 3-keto derivative (Bernaerts & De Ley, 1960*a*, 1961). Furthermore, Grebner, Durbin & Feingold did not state whether the micrococcus had its habitat in or on plant galls.

Agrobacterium rubi. According to *Bergey's Manual* (1957) the difference between *Agrobacterium rubi* and *A. tumefaciens* is based only on a few minor features (nitrite and indole production, rate of growth) and one major one (*A. rubi* infects only members of the genus *Rubus*). The following arguments show that it is unnecessary to keep *A. rubi* as a separate species and that it would be better to include it in *A. tumefaciens*. (*a*) A slight difference in nitrite and indole production and a slower rate of growth are of little value for species differentiation. (*b*) The following examples show that organisms labelled *A. rubi* are not specifically pathogenic for *Rubus* plants. Our strain is markedly pathogenic for tomato and Datura (this paper) and for sugar beet (Starr, 1946). Other strains are virulent for pinto bean (Drs Lippincott & Heberlein, personal communication). Typical '*tumefaciens*' strains are also pathogenic for these plants. McKeen (1954) also pointed out that it was almost impossible to distinguish between *A. rubi* and *A. tumefaciens*. (*c*) Transformations with DNA can be made between these two species (Klein & Klein, 1953), showing the close similarity between their chromosomes. (*d*) The % GC and the σ value of *A. rubi* DNA is nearly indistinguishable from the values for DNAs of the '*tumefaciens*' strains; these results are not opposed to the inclusion *A. rubi* into the '*tumefaciens*' group.

Agrobacterium pseudotsugae may be a species of *Agrobacterium*, but at present it is a doubtful case. The only strain available to us, NCPPB 180, had a T_m value of 97·1° and a very narrow compositional distribution of its DNA molecules. This shows that its chromosome has very little in common with *Agrobacterium* proper ('*radiobacter*' '*tumefaciens*', '*rhizogenes*'). Our strain was doubtfully tumourigenic for tomato, did not produce 3-ketolactose and its inclusion in *Agrobacterium* was already

doubted by Knösel (1962*a*). Two other strains were avirulent for pinto beans (Drs Lippincott & Heberlein, personal communication). More research on this group is required.

No decision can be taken about the taxonomic position of the strain *Agrobacterium tumefaciens* NCPPB 930. At best it might be a 3-ketolactose-negative *A. radiobacter* or *A. rhizogenes*.

Strains which are excluded from the genus Agrobacterium

Agrobacterium stellulatum was included in this genus because of one property only, namely that it forms star-shaped clusters. This property occurs with many genera, mostly tumour- or nodule-forming ones, such as *Agrobacterium, Rhizobium, Pseudomonas, Chromobacterium, Phyllobacterium* (Knösel, 1962*a,b*). Except for star-formation, these *A. stellulatum* organisms do not display any of the characteristics of *Agrobacterium*. It was reported to be polarly flagellate by Stapp & Knösel (1954); this was confirmed in the present work. It grows preferentially on sea-water media. We found it to be non tumourigenic for tomato and Datura, not to produce 3-ketolactose, and to have a T_m value of 96·5° which is decidedly out of the common range of the other strains. This organism should therefore be removed from the genus *Agrobacterium*; its flagellation, growth in sea-water media, T_m value and variance σ are not opposed to its inclusion in the marine pseudomonads. It may be that the strain '*stellulatum*' is closely related to the polarly flagellated *Pseudomonas savastanoi, P. tonelliala* and *P. rimaefaciens*; these also form stars (Knösel, 1962*a*).

'*Bacterium gypsophilae*' (Brown, 1934) was included in *Agrobacterium* mainly because of its tumourigenesis (Starr & Weiss, 1943). The strain '*gypsophilae*' NCPPB 179 is probably not an Agrobacterium because it has the following aberrant properties: (*a*) its T_m of 92·4° is completely out of the range of the other agrobacteria; the variance σ is unusually wide, 2·5 as against a range of 0–0·88 for the other strains; (*c*) this strain has an unusually high content of DNA, whereas the yield from species of *Agrobacterium* proper is rather poor; (*d*) our strain has a yellow pigment, in agreement with the original description (Brown, 1934), while other agrobacteria are not pigmented; (*e*) a strain of '*gypsophilae*' was not phytopathogenic for *Gypsophila paniculata* (Knösel, 1962*a*). The exact taxonomic position of the strains of '*gypsophilae*' remains to be established. Its low T_m value excludes its classification in the genus *Xanthomonas* and thus disagrees with Prévot's (1961) proposal; all xanthomonads have a T_m range from 95·4° to 97·6° (De Ley & Van Muylem, 1963; De Ley, Park, Tijtgat & Van Ermengem, 1965).

The strain labelled '*tumefaciens*', ATCC 11095, being polarly flagellate, cannot be included in the genus *Agrobacterium*. It was found to be non-pathogenic in the present work and by Drs Lippincott & Heberlein (personal communication).

An improved definition of and classification within the genus Agrobacterium

By combining the above results with the commonly known morphological and physiological features of these organisms an improved definition of and classification within the genus *Agrobacterium* is now possible. A temporary proposal may be as follows: Gram-negative, small short rods, motile by peritrichous flagella of number frequently up to 5–6. No detectable gas, and no or slight acid production

on ordinary media. No pigmented strains known. Optimal growth temperature 25–30°. Frequently pathogenic (hypertrophies). Many strains produce 3-ketolactose.

Key: I. Frequently pathogenic for angiosperms. T_m in the range 94·5° ± 0·8°, variance σ not above 1.

A. *Agrobacterium radiobacter*: non-pathogenic.
A. radiobacter var. *tumefaciens*: crown-gall bacteria.
Many strains of this species produce 3-ketolactose.

B. *A. rhizogenes*: hairy-root disease.

II. Pathogenic for gymnosperms. T_m about 97°.
A. pseudotsugae.

The reasons for including both *A. radiobacter* and *A. tumefaciens* in one species are as follows. (*a*) The values of T_m and of σ of both groups of organisms are indistinguishable, indicating a possible near-identity of their chromosomes. (*b*) DNA transforms a non-pathogenic *Agrobacterium radiobacter* into a pathogenic strain (Klein & Klein, 1953, 1956). (*c*) Artificial loss of pathogenicity in *Agrobacterium tumefaciens* can be induced by repeated subcultivation in amino-acid containing-media (Stapp, 1958, p. 93), or by ultraviolet irradiation (Béaud, Manigault & Stoll, 1963). (*d*) Both types are often lysed by the same phages (Roslycky, Allen & McCoy, 1962, 1963). (*e*) They are antigenically identical in the S phase (Coleman & Reid, 1945). (*f*) Numerical analysis reveals their identity; Graham (1964) even proposed to include both groups in *Rhizobium radiobacter*. If the proposal to include both species in one be accepted, it must be called *A. radiobacter* for historical reasons; its tumourigenic variety would then become *A. radiobacter* var. *tumefaciens*.

It is possible that *Agrobacterium rhizogenes* is also only a variety of *A. radiobacter*. Until this has been investigated further it seems advisable to keep *A. rhizogenes* as a separate species centre.

Organisms related to Agrobacterium

In *Bergey's Manual* (1957) the family Rhizobiaceae comprises three genera: *Rhizobium*, *Agrobacterium*, *Chromobacterium*. On the other hand, some authors assert that there is little or no relation between *Agrobacterium* and *Rhizobium*. Prévot (1961) separated the genera and put *Agrobacterium* between such unrelated groups as the Nitrobacteriae and the Thiobacteriaceae. Krassilnikov (1959), by including the agrobacteria in *Pseudomonas*, kept them removed from *Rhizobium*. The super-generic classification of these organisms is thus a matter of personal preference and not necessarily the reflexion of the natural relationship. The latter, however, can now be fixed by numbers. Ideally this should be possible by DNA-hybridization; for want of these data we attempted it by combining two numerical parameters: (i) the mean % similarity $\bar{S}$ derived from taximetric analysis (a quantification of phenotypic relationship) as published in the literature (ref. see Fig. 2); (ii) the % GC (a measure of their possible genetic relationship), from data obtained in the senior author's laboratory. The groups involved are:

(1) *Beijerinckia*,

(2) *Rhizobium leguminosarum*,

(3) *Agrobacterium* (or *Rhizobium*) *radiobacter*, *Agrobacterium* (or *Rhizobium radiobacter* var. *tumefaciens* (may or may not include the *rhizogenes* strains),

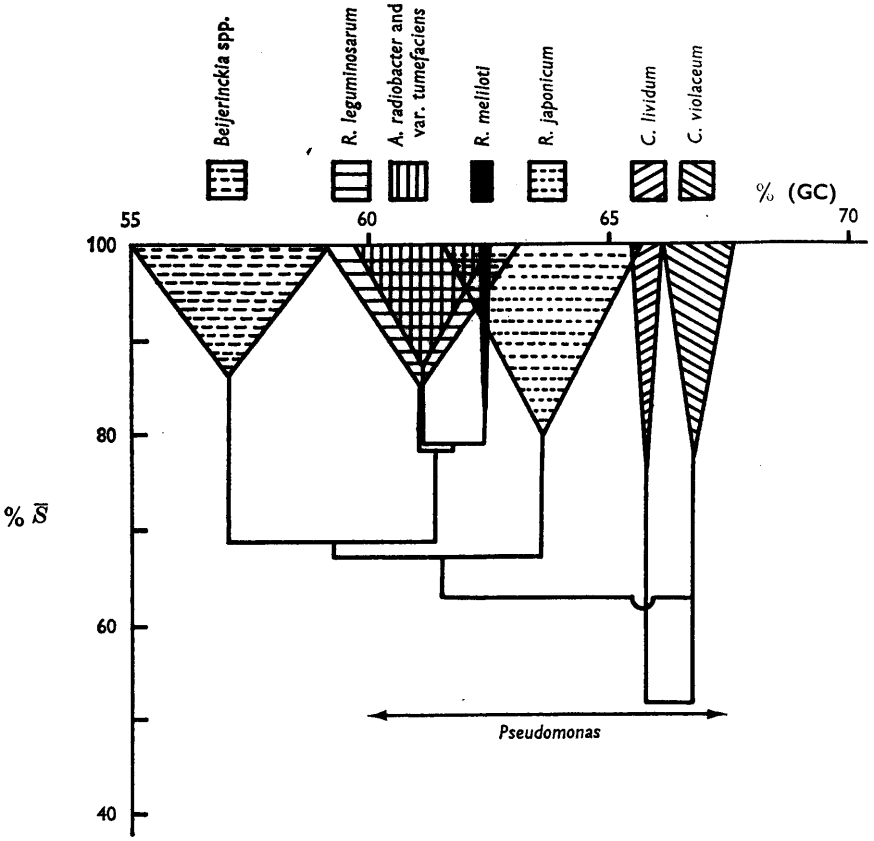

Fig. 1. Relationship between agrobacteria and some other organisms based on a genotypic (DNA base composition) and a phenotypic (mean % similarity $\bar{S}$) parameter.

Every group is represented by an inverted triangle, the top line representing the range of % GC and the bottom tip being the $\bar{S}$ value, arbitrarily put in the middle of the % GC range. Thus, strains of *Rhizobium japonicum* have a % GC ranging from 62 to 65·5 and an intra-group $\bar{S}$ value of 80. The $\bar{S}$ values of the chromobacteria were taken from Sneath (1957); 105 features of 20 strains of *Chromobacterium lividum* and 18 strains of *C. violaceum* were involved. A random selection was made of 7 lividum strains and 6 violaceum strains from Sneath's collection and used for the % GC determination (De Ley, 1964; De Ley & Van Muylem, 1963). The strains *C. violaceum* 9373 and *C. lividum* GA appear to be aberrant and are not included in the graph. The taximetric analysis by Graham (1964) involved 100 properties of 8 *Beijerinckia* strains, 32 strains of *Rhizobium leguminosarum*, 18 strains of *Agrobacterium radiobacter* and *A. tumefaciens*, 25 strains of *R. meliloti* and 27 strains of *R. japonicum*. We calculated the inter- and intra-group $\bar{S}$ values from this author's results. For the % GC determination 3 strains of *Beijerinckia* (De Ley & Park, 1966; De Ley, unpublished), 18 strains of *R. leguminosarum*, 2 of *R. meliloti* and 15 of *R. japonicum* (De Ley & Rassel, 1965), and 36 agrobacteria (this paper) were used. Although we did not use Graham's strains for the determination of % GC, yet our strains belong to the same named groups because: (i) they are culturally, morphologically, physiologically and pathogenically identical; (ii) Graham's taxonomic conclusions based on taximetric analysis, and ours based on % GC, are basically identical. Therefore the position of the triangles for these groups is only a close approximation and may undergo slight shifting when the same strains are studied by both methods. In order not to obscure the graph and as a measure of comparison, the % GC values of the pseudomonads (De Ley, Park, Tijtgat & Van Ermengem, 1965) are represented separately as a horizontal bar.

(4) *Rhizobium meliloti*,
(5) *Rhizobium* (or *Phytomyxa*) *japonicum*,
(6) *Chromobacterium* (or new generic name to be decided later) *lividum*,
(7) *Chromobacterium violaceum*.

Agrobacterium, *Rhizobium*, *leguminosarum* and *R. meliloti* all have a % GC value within the same range. Klein & Klein (1953) showed that the pathogenicity of *Agrobacterium tumefaciens* can be transfered to *Rhizobium leguminosarum*; similar results were obtained by Manil (1960). These results indicate a great similarity between the chromosomes of the two groups. As another phenotypic argument it may be mentioned that a strain of *R. leguminosarum* produces some 3-ketolactose from lactose (Bernaerts & De Ley, unpublished results). The inter-group mean similarity $\bar{S}$ of these organisms is high (Graham, 1964) and was calculated from this author's results to be about 79.

Beijerinckia indica is next closely related to the above groups, closely followed by *Rhizobium japonicum*.

Chromobacterium. The definition of this genus differs from one text to the other. *Bergey's Manual* (1957) considers only a few violet and bluish organisms and includes this genus in Rhizobiaceae. Prévot (1961) considered the chromobacteria in the same narrow sense as *Bergey's Manual* but removed them completely from both the agrobacteria and the rhizobia. In Krassilnikov (1959) Chromobacterium is only a collective noun, including a great variety of pigmented strains; it is, however, not a biological entity. We consider here only the violacein-producing biotypes *Chromobacterium lividum* and *C. violaceum* which are clearly separated both taximetrically (Sneath, 1957) and by their % GC (De Ley, 1964; De Ley & Van Muylem, 1963). Fig. 1 stresses Sneath's (1957) presumption that both groups deserve separate generic status. The production of violacein by both groups probably signifies (at least if the biosynthetic pathway for the pigment is the same in each) that in the course of evolution the pigment-producing system was formed first, followed only later by mutational diversification into the lividum and violaceum groups. From Graham's (1964) results, it can be calculated that *C. violaceum* shares an average of 60–66 % $\bar{S}$ with the other groups.

An unbiased opinion on the existence and the limits of a family Rhizobiaceae cannot be given; this may be more a matter of nomenclature than of classification. A more satisfactory taxonomic relationship is represented graphically in a plot of similarity *versus* DNA base composition.

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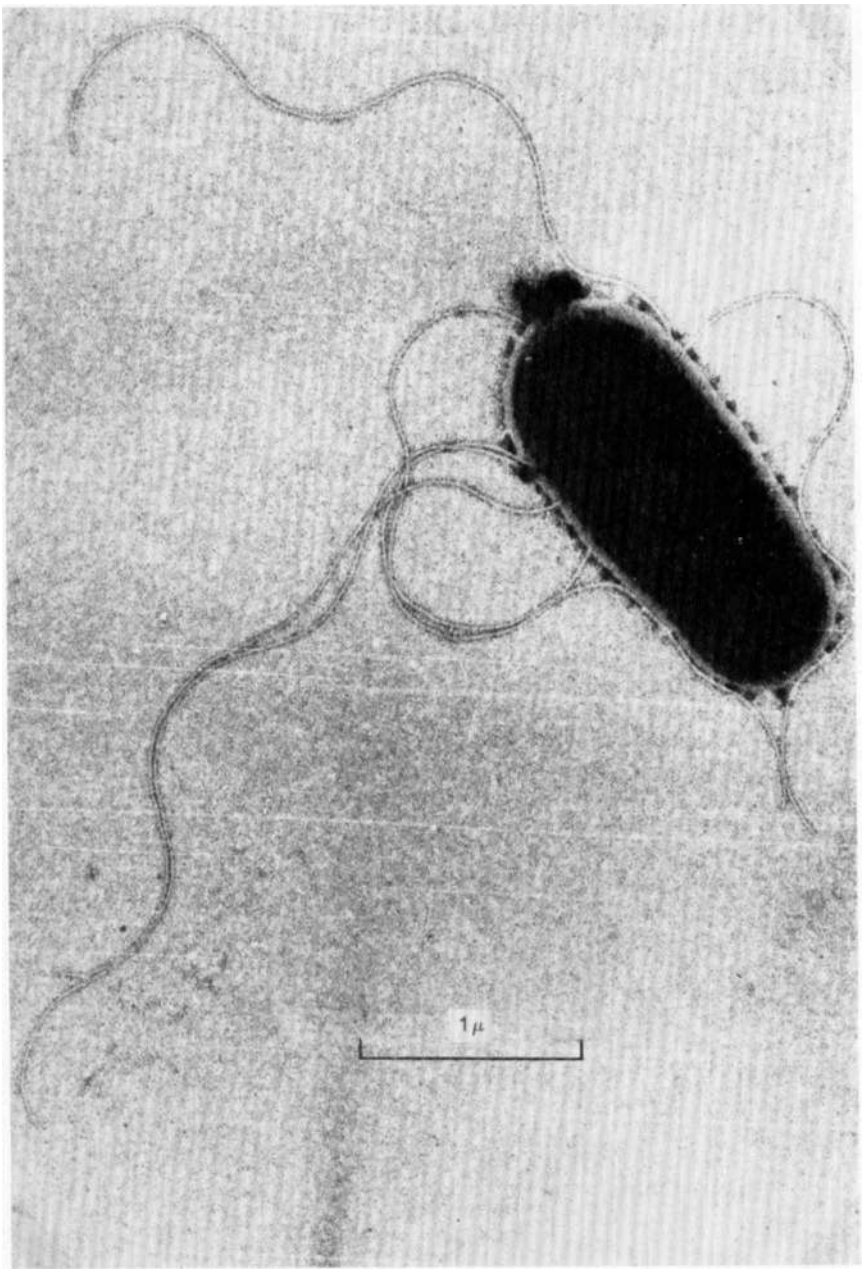
EXPLANATION OF PLATE

Electron micrograph of *Agrobacterium radiobacter* var. *tumefaciens* strain c, showing the type of peritrichous flagellation.



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