Deoxyribonucleic Acid Homology and Taxonomy of *Agrobacterium*, *Rhizobium*, and *Chromobacterium*

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Hybridization experiments were carried out between high molecular weight, denatured, agar-embedded deoxyribonucleic acid (DNA) and homologous, nonembedded, sheared, denatured ¹⁴C-labeled DNA from a strain of *Agrobacterium tumefaciens* and *Rhizobium leguminosarum* (the reference strains) in the presence of sheared, nonembedded, nonlabeled DNA (competing DNA) from the same or different nomen-species of *Agrobacterium*, *Rhizobium*, *Chromobacterium*, and several other organisms. Percentage of DNA homology was calculated from the results. The findings are discussed in relation to previous taximetric studies, present classification schemes, and guanine-cytosine content of the DNA. Strains of *A. tumefaciens*, *A. radiobacter*, *A. rubi*, *A. rhizogenes*, *R. leguminosarum*, and *R. meliloti* exhibited a mean percentage of DNA homology greater than 50 with the two reference strains. *A. tumefaciens*, *A. radiobacter*, and *A. rubi* were indistinguishable on the basis of DNA homology, with strain variations for this group involving up to 30% of their base sequences. The remainder of the organisms studied fall into at least six distinct genetic groups: (i) *R. (Agrobacterium) rhizogenes*, which is more homologous to *R. leguminosarum* than to the *A. tumefaciens*-*A. radiobacter* group; (ii) *R. leguminosarum*; (iii) *R. meliloti*; (iv) *R. japonicum*, which has a mean DNA homology of some 38 to 45% with the reference strains; (v) *Chromobacterium*, which is as genetically remote from the reference strains as, for example, *Pseudomonas*; and (vi) *A. pseudotsugae* strain 180, which has a DNA homology with *A. tumefaciens* and *R. leguminosarum* of only about 10%. Since this latter homology value is similar to what was found after hybridizations between the reference strains and organisms such as *Escherichia coli* and *Bacillus subtilis*, *A. pseudotsugae* should definitely be removed from the genus.

Bacteria within the genera *Agrobacterium* and *Rhizobium* have the unique capacity to induce such phenomena as prolific root formation, nitrogen-fixing root nodules, and autonomous crown-gall tumors on many higher plants. In addition to their obvious agricultural importance, these bacteria have become very useful tools in attempts to understand better the underlying mechanisms of plant morphogenesis, phytopathogenicity, and host-parasite relationships (12, 15, 18, 26). Surprisingly little, however, is known about the genetic interrelationships of the agrobacteria and rhizobia. The taxonomy of these bacteria is also confused. Their classification is frequently based upon only a few phenotypic properties, resulting in considerable variation from one text to another (11, 17, 22, 27;

Bergey's Manual). In *Bergey's Manual*, for example, where the genera *Agrobacterium*, *Rhizobium*, and *Chromobacterium* are considered as taxonomically related by virtue of their being grouped together in the family *Rhizobiaceae*, the distinction among the different species and genera rests on such qualitative criteria as nitrate reduction and pathogenic host range, which at best represent only a minute portion of the total bacterial genome. Only recently have attempts been made to clarify the nature and validity of the relationship among the bacteria in the above three genera by means of more quantitative approaches. The need for extensive taxonomic revision and further study has been emphasized by the taximetric analysis of the chromobacteria by Sneath (24) and of the agrobacteria, rhizobia, and chromobacteria by Graham (13), as well as investigations by De Ley and co-workers (5, 6, 9) on the guanine-cytosine (GC) content of the deoxyribonucleic acid (DNA) of these bacteria.

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Taximetric analysis, though usually based upon 100 or more phenotypic properties, still takes into account only a small portion of the total genome of the bacterium (8) and, therefore, does not necessarily represent the actual amount of genetic information that is common among strains. Because genetic similarity can be measured by means of the DNA hybridization technique (14, 20) and because such knowledge could prove useful in clarifying the taxonomy of these bacteria and in showing possible correlations between infectivity and the presence of a "common DNA part," DNA hybridization experiments have been carried out.

MATERIALS AND METHODS

Organisms. Several strains from most of the nomenclature species of *Agrobacterium*, *Rhizobium*, and *Chromobacterium* were selected on the basis of authenticity and representative GC values, and are listed in Table 1. Those bacteria bearing the designation ATCC were purchased from the American Type Culture Collection (Washington, D.C.). Strains having the prefixes NCPPB, ICPB, NCTC, USDA, and MRC were kindly donated by the National Collection of Plant Pathogenic Bacteria (Ministry of Agriculture, Fisheries, and Food, Hatching Green, Harpenden, England), the International Collection of Phytopathogenic Bacteria (Department of Bacteriology, University of California, Davis), the National Collection of Type Cultures (London, England), L. W. Erdman (U.S. Department of Agriculture, Beltsville, Md.), and P. H. A. Sneath (Medical Research Council, Mill Hill, London, England), respectively. Strains B6, 1.5, 3.2, and S1005 were obtained through the courtesy of C. Bonnier, Institut Agronomique de l'Etat, Gembloux, Belgium. Strain 8196U was isolated in this laboratory.

Cultivation of unlabeled bacteria. All bacteria except those used in the preparation of ^{14}C -labeled DNA were grown in Roux flasks on agar-solidified media for about 2 days at 27 C. The agrobacteria were grown on a medium containing (w/v): 1% glucose, 1% yeast extract (Nederl. Gist-en Spiritusfabriek, Brugge, Belgium), 0.1% $(\text{NH}_4)_2\text{SO}_4$, 0.025% KH_2PO_4 , and 2.5% agar. A medium containing (w/v) 1% glucose, 1% yeast extract, 2% CaCO_3 , and 2.5% agar was used for growing the chromobacteria. Strains of *R. leguminosarum* and *R. meliloti* were grown on a medium containing (w/v): 1% yeast extract, 0.05% K_2HPO_4 , 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02% NaCl , 0.02% CaCl_2 , 0.0002% FeCl_3 , 2.5% agar. To reduce polysaccharide production, strains of *R. japonicum* were grown on a medium containing (w/v): 0.46% glycerol, 0.1% L-arabinose, 0.1% yeast extract, 0.1% K_2HPO_4 , 0.13% CaSO_4 , 0.07% KNO_3 , 0.0004% FeCl_3 , 0.036% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The bacteria were harvested with saline-EDTA (0.15 M NaCl and 0.1 M ethylenediaminetetraacetate, pH 8), centrifuged, washed by intermittent centrifugation and resuspension in saline-EDTA to remove polysaccharides, and lyophilized. The dried cells were stored at 4 C until used for the preparation of DNA.

Cultivation of ^{14}C -labeled bacteria. Klett flasks (1-liter) containing 200 ml of appropriate liquid medium (see above) and 0.2 mc (20 ml) of filter-sterilized uracil-2- ^{14}C (The Radiochemical Center, Amersham, Buckinghamshire, England) were inoculated with ca. 10^6 cells of either *A. tumefaciens* strain B6 or *R. leguminosarum* strain 316C10A. The cultures were incubated on a reciprocal shaker at 27 C. Growth was followed with a Klett colorimeter, and at the end of the log phase the bacteria were harvested by centrifugation, washed twice with saline-EDTA, and lyophilized. The ^{14}C -labeled DNA was then extracted from the dried cells.

DNA preparations. All DNA was extracted and purified according to the procedure of Marmur (21), modified so as to include a deproteinization step with pronase (40 $\mu\text{g}/\text{ml}$; Calbiochem, Los Angeles, Calif.) after ribonuclease treatment. This procedure typically yielded DNA with a molecular weight between 5×10^6 and 10×10^6 , as determined by standard analytical ultracentrifugation techniques. Purified ^{14}C -labeled DNA was dissolved in $0.2 \times \text{SSC}$ buffer ($1 \times \text{SSC}$ buffer = 0.15 M NaCl and 0.015 M trisodium citrate, pH 7), sheared in a French pressure cell at 12,000 psi to an average molecular weight of about 3×10^6 , denatured by heating for 6 min at 106 C in a glycerol bath, immediately cooled in an ice bath, and adjusted to a final concentration of 50 $\mu\text{g}/\text{ml}$ in $2 \times \text{SSC}$ buffer. Purified, precipitated, unlabeled DNA was dissolved to a concentration of about 5 mg/ml in $0.1 \times \text{SSC}$ buffer and stored at 4 C with a few drops of chloroform until used for the preparation of "competing DNA" and DNA-agar. Competing DNA was prepared by shearing and denaturing the DNA as above and adjusting the SSC buffer concentration to 2. DNA-agar was made according to the procedure of Bolton and McCarthy (3) with the exception that it was heated at 106 C in $0.1 \times \text{SSC}$ instead of at 100 C because of the high melting point of these DNA preparations.

Hybridizations. DNA hybridization was carried out by use of the technique of McCarthy and Bolton (20) as modified by Hoyer et al. (14). In essence, 30 μg of sheared, denatured, ^{14}C -labeled DNA (600 to 800 counts per min per μg) from either *A. tumefaciens* strain B6 or *R. leguminosarum* strain 316C10A was incubated with 300 μg of high molecular weight unlabeled, denatured, agar-embedded DNA from the same strain. Incubation was for 16 hr at 60 C in a total volume (milliliters) of $2 \times \text{SSC}$ buffer amounting to 2 to 2.5 times the weight (grams) of the DNA-agar depending upon the concentration of DNA in the DNA-agar preparation used. The above hybridization procedure was carried out both in the presence and absence of 2,000 μg of competing DNA (sheared, denatured, unlabeled, non-agar-embedded DNA) from the same strain or different strains. Appropriate controls were run for each new preparation of DNA-agar. After incubation, the hybridization mixture was added to a jacketed column having a sintered-glass base covered by filter paper and glass wool. The DNA-agar in the column was then washed with $2 \times \text{SSC}$ buffer at 60 C to elute the unhybridized fraction of ^{14}C -DNA (about 120 ml at a flow rate of 100 ml/hr).

TABLE 1. DNA base composition and DNA homology among strains of *Agrobacterium*, *Rhizobium*, and *Chromobacterium*

Organism	Strain no.	Per cent GC	Per cent DNA homology with	
			<i>A. tumefaciens</i> B6	<i>R. leguminosarum</i> 316C10A
<i>A. tumefaciens</i>	ICPB TT111	60.6	77	59
	ATCC 11.156	61.1	71	39
	B6	61.4	100	65
	NCPPB 4	61.4	70	52
	ATCC 4720	62.1	86	49
	NCPPB 397	62.2	100	52
	8196U	—	92	70
Mean		61.5	85	55
<i>A. radiobacter</i>	ICPB TR-1	61.2	93	62
	S1005	61.3	70	56
	ATCC 4718	61.8	82	58
	ICPB TR-6	62.1	87	53
Mean		61.6	83	57
<i>A. rubi</i>	ICPB TR-2	59.6	70	59
<i>A. rhizogenes</i>	ICPB TR-101	61.1	58	71
	ICPB TR-107	62.3	55	59
	ICPB TR-7	62.8	44	64
Mean		62.1	52	65
<i>A. pseudotsugae</i>	NCPPB 180	67.7	18	6
<i>R. leguminosarum</i>	USDA 316C10A	61.4	52	100
	USDA 3F3C1	62.5	52	84
Mean		62.0	52	92
<i>R. meliloti</i>	USDA 3D0a30	62.3	40	52
	1.5	62.5	48	65
Mean		62.4	44	59
<i>R. japonicum</i>	USDA 311b59	64.0	40	38
	3.2	64.8	38	45
Mean		64.4	39	42
<i>C. lividum</i>	MRC MB	66.1	35	36
	MRC RU	65.5	25	34
	MRC GA	71.4	38	38
Mean		67.6	33	36
<i>C. violaceum</i>	NCTC 9371	66.1	28	42
	NCTC 8685	66.9	35	40
	NCTC 8684	67.0	34	34
	NCTC 9757	67.1	33	30
Mean		66.8	33	37
<i>Pseudomonas^a fluorescens</i>	488	62.4	40	38

TABLE 1—Continued

Organism	Strain no.	Per cent GC	Per cent DNA homology with	
			<i>A. tumefaciens</i> B6	<i>R. leguminosarum</i> 316C10A
<i>Acetobacter</i> ^a <i>liquefaciens</i>	9505	64.3	31	41
<i>Azomonas</i> ^a <i>macrocytogenes</i>	8200	58.6	28	48
<i>Escherichia</i> ^a <i>coli</i>	B	52.5	14	19
<i>Bacillus</i> ^a <i>subtilis</i>	BQ2	47.7	3	10

^a Experiments by Park (Ph.D. Thesis, State University of Gent, Gent, Belgium, 1966), carried out in conjunction with the present investigation.

The ¹⁴C-labeled DNA that was bound to the high molecular weight DNA in the agar was then eluted by washing with 90 ml of 0.01 × SSC buffer at 75 C. The degree of hybridization was determined by comparing the amount of ¹⁴C-label which had annealed to the agar-bound DNA with that which was washed free. The percentage of homology between the DNA of the reference strains and the DNA from the various other strains (competing DNA) was calculated from their relative effect upon the amount of ¹⁴C-labeled DNA which, in their presence, hybridized with the agar-bound reference DNA. DNA homology values were typically reproducible to within 1 to 6%. Hybridizations were usually repeated two to three times, and the values given represent the mean of the separate determinations. The competition procedure was chosen because it eliminated the error associated with making DNA-agar from different DNA preparations.

Radioactivity determinations. Disintegrations were counted as previously described by De Ley and Friedman (7). The correction factor for self-absorption in 2 × SSC buffer under the conditions used was 14.4%; for 0.01 × SSC buffer, quenching was negligible.

GC content. DNA base composition was determined by thermal denaturation as described by De Ley and co-workers (9, 10).

RESULTS AND DISCUSSION

The different bacteria used in this study, along with their GC content, and DNA homology with *A. tumefaciens* strain B6 and *R. leguminosarum* strain 316C10A are listed in Table 1.

With the exception of *A. pseudotsugae* and *R. japonicum*, the agrobacteria and rhizobia have about the same range of GC content, mainly, 59 to 63% of their DNA. *R. japonicum* and the chromobacteria typically have a GC value of around 64 and 66%, respectively. Far more extensive studies by De Ley and co-workers (6, 9) suggest that these are representative values.

The genetic relationships among the different strains studied are depicted in Fig. 1. The difference between *A. tumefaciens* and *A. radiobacter* resides mainly in the tumor-forming capacity of the former. It is impossible to distinguish between

these two strains on the basis of DNA homology. Strains of *A. radiobacter* exhibit the same range of homologies to strain B6 as do the pathogenic strains and, therefore, should have the same species designation. Such a move would be in accordance with the proposals of Graham (13) and De Ley et al. (6), which were made after an examination of the phenotypic and molecular properties of these bacteria.

Although definite conclusions cannot yet be drawn about the taxonomic status of *A. rubi* from the single strain studied, it would appear on the basis of its homology with the reference strains that it, too, should be placed in the same species with *A. radiobacter* and *A. tumefaciens*. The limited criteria upon which the species designation of *A. rubi* is based has already been emphasized (6). The belief that bacteria within this species are pathogenic only to plants in the genus *Rubus* has been disproven a number of times both in this laboratory (6) and in others (18, 25).

The DNA from strain 180 of *A. pseudotsugae* was only slightly similar to that of the reference strains, its GC content was high, and its pathogenicity was questionable. These observations clearly indicate that strain 180 is not a valid *Agrobacterium* strain and that it should be removed from this genus. This agrees very well with the consensus (16; Second International Conference on Phytopathogenic Bacteria, Lisbon, 1967) that the taxonomic status of *A. pseudotsugae* should be reinvestigated and revised.

Unexpectedly, strains of *A. rhizogenes* show a higher degree of homology with *R. leguminosarum* than with *A. tumefaciens*. This finding is in agreement with recent studies by Kersters (*personal communication*) on the particulate dehydrogenases of bacteria in the *Agrobacterium-Rhizobium* group. *A. rhizogenes*, like the rhizobia, contain a greater abundance of particulate enzymes active on saccharides than does the *A.*

radiobacter-*A. tumefaciens* group. Although *A. rhizogenes* and *R. meliloti* exhibit about the same degree of DNA homology with *R. leguminosarum*, the question of whether they are similar by virtue of the same portion of their genomes is presently under investigation.

The three species of *Rhizobium* fall within distinct genetic groups in their relation to the *R. leguminosarum* reference strain, thus confirming their species status and the findings of Graham (13) and De Ley (9).

In contrast to the other valid agrobacteria and rhizobia, strains of *R. japonicum* exhibit only about as much DNA homology with both reference strains as do such genera as *Acetobacter*, *Azomonas*, and *Pseudomonas*. The implications of these findings are discussed later in the text.

C. lividum and *C. violaceum* are also far removed genetically from the bulk of the *Agrobacterium*-*Rhizobium* group. They should therefore be reclassified in a manner which does not exaggerate their actual relationship to the agrobac-

FIG. 1. Schematic representation of the genetic relationships of the different strains of bacteria examined with *Agrobacterium tumefaciens* strain B6 and *Rhizobium leguminosarum* strain 316C10A (heavy lines). All data were taken from Table 1. Those organisms having a mean of less than 50% of their DNA homologous to both strains B6 and 316C10A are plotted as the average of their mean percentage of homology with both strains. Those strains having a mean percentage of DNA homology greater than 50% with either strain B6 or strain 316C10A are plotted against the strain to which they are most homologous. The point at which strain B6 and strain 316C10A are plotted as separate lines (52%) represents the mean percentage of homology that the "B6-like" strains share with the "316C10A-like" strains and vice versa.

teria and rhizobia as is presently the case in *Bergey's Manual*. We suggest that they be removed from the family *Rhizobiaceae*. Whether this family grouping has any real meaning has already been questioned (6).

The *Rhizobiaceae* is one of the few groups of bacteria in which both taximetric analysis and DNA homology studies have been carried out and, thus, provides an interesting opportunity to compare the results obtained by these rather different techniques. Figure 2 shows a plot of the mean percentage of taximetric similarity against the mean percentage of DNA homology. Greater resolution was obtained by the hybridization technique, which expands the differences over a 67% homology range, as compared with taximetric analysis, which extends only over a 38% similarity range. The farther removed two strains are from each other genetically, the less agreement there is between both methods. At least two explanations might account for this discrepancy. First, taximetric analysis involves probably not more than one-fifth of the genome; and second, hybridization experiments will show lack of homology among cistrons which code for enzymes producing similar phenotypic properties, by virtue of a common active center, but

differing in amino acid sequence or content (or both).

With the exception of one nomen-species, there is a good qualitative correlation between the results obtained by DNA hybridization and taximetric analysis in this particular case. The very high taximetric similarity observed between strains of *R. meliloti* and *A. tumefaciens* was not reflected in their DNA homology. This high value might be a consequence of the particular properties selected for analysis and, if so, would be expected to diminish as more properties are examined.

It may be noticed from Fig. 2 that percentage of DNA homology is not higher than percentage of phenotypic similarity. There is thus no indi-

TABLE 2. Comparison between the orthodox and a proposed classification scheme for the agrobacteria and rhizobia based upon DNA homology, taximetric similarity (13), DNA base composition (6, 9), and flagellation (6, 9)

Classification according to <i>Bergey's Manual</i>	Proposed classification
<i>Rhizobium leguminosarum</i>	<i>Rhizobium</i> Frank, 1889 <i>leguminosarum</i> (Frank) Frank, 1889 (type species)
<i>trifolii</i>	<i>leguminosarum</i>
<i>phaseoli</i>	<i>leguminosarum</i>
<i>meliloti</i>	<i>meliloti</i> Dangeard, 1926
<i>japonicum</i>	<i>japonicum</i> (Kichner) Buchanan, 1926
<i>lupini</i>	<i>japonicum</i>
cowpea group	<i>japonicum</i>
<i>Agrobacterium radiobacter</i>	<i>Rhizobium radiobacter</i> ^a (Beijerinck and van Delden) Pribram, 1933
<i>tumefaciens</i>	<i>radiobacter</i> var. <i>tumefaciens</i>
<i>rubi</i>	<i>radiobacter</i> var. <i>tumefaciens</i>
<i>rhizogenes</i>	<i>rhizogenes</i> (Riker et al.) <i>comb. nov.</i>
<i>pseudotsugae</i>	Taxonomic position unknown
<i>gypsophila</i>	Taxonomic position unknown (6)
<i>stellulatum</i>	<i>Pseudomonas</i> ? (6)

^a Graham's (13) combination of *R. radiobacter* with variety *tumefaciens* is justified according to Rule 19, International Code of Nomenclature of Bacteria and Viruses. Indeed, the species *Bacillus radiobacter* was validly and legitimately described by Beijerinck and van Delden (1), and the description was extended by Löhnis (19) before the description of *Bacterium tumefaciens* by Smith and Townsend (23).

FIG. 2. Relationship between DNA homology and taximetric similarity of bacteria within the *Rhizobiaceae*. The mean percentage of similarity between the different taxospecies and either *Agrobacterium tumefaciens* or *Rhizobium leguminosarum* was calculated from Graham's (13) data. These values are plotted against the mean percentage of DNA homology between the same species and either *A. tumefaciens* strain B6 or *R. leguminosarum* strain 316C10A. The latter data are taken from Table 1.

TABLE 3. Characteristic properties of *Rhizobium* geno-species

Features	<i>R. radiobacter</i>	<i>R. radiobacter</i> var. <i>umejactens</i>	<i>R. rhizogenes</i>	<i>R. leguminosarum</i>	<i>R. meliloti</i>	<i>Rhizobium japonicum</i>
3-Ketolactose from lactose (2, 6) Flagellation (6, 9)	Usually + Sparse peritrichous often with 1 sub-polar None	Usually + Sparse peritrichous often with 1 sub-polar Crown gall	— Sparse peritrichous often with 1 sub-polar Root induction	— Sparse peritrichous often with 1 sub-polar Root nodule	— Sparse peritrichous often with 1 sub-polar Root nodule	— Single subpolar Root nodule
Parasitic properties or phytopathogenicity						
Cross-inoculation groups (12) which comprise the geno-species				II, III, IV, V, VIII, usually IX-XXII	I	V, VI, VII, rarely IX-XXII
DNA base composition in per cent GC (6, 9)	59.5 to 63.0	59.5 to 63.0	61.0 to 63.0	59.0 to 63.5	About 62	61.5 to 65.5
Growth properties	Good growth on Na-selenite agar —	Good growth on Na-selenite agar —	No growth on Na-selenite agar +	Fast growth on mannitol agar	Fast growth on mannitol agar	Slow growth on mannitol agar
Particulate dehydrogenases for sugars (Kerstens, <i>to be published</i>)						

cation that homologous, phenotypically non-expressed DNA (unused genes) would be present in these chromosomes. Similar conclusions have been reached for *Pseudomonas* (10).

The DNA hybridization technique, because it takes into account the entire genome, offers a sensitive and quantitative approach to the classification of organisms. It seems feasible, therefore, that it should be used to clarify the presently ill-defined concept of bacterial genus and species. It can be seen from the present study that authentic strains for the same nomen-species (see *A. tumefaciens*, Table 1 and Fig. 1) display at least a 70% DNA homology. In view of the results with the *A. radiobacter* and *A. rubi* strains, it seems that this degree of homology would be a suitable boundary for a genetic species. In a comprehensive study of DNA homology among a number of *Xanthomonas* strains (8), a similar value was suggested for the limit of the genospecies.

Figure 1 presents the taxonomist with an interesting nomenclatorial problem of where to draw the genus line. If the line is drawn between the 50 to 70% homology level, then *Agrobacterium* remains a genus independent from *Rhizobium*, but *A. rhizogenes* and *R. meliloti* also have to become separate genera, and *a fortiori* *R. japonicum*. It seems more realistic to draw the line at about 50% homology. This latter value would also correspond with that which was found to be a suitable genus boundary for *Pseudomonas* (10). Table 2 gives a proposed classification scheme that would be in line (except for *R. japonicum*) with the above findings. For comparison, the orthodox classification as it stands in *Bergey's Manual* is also included. In Table 3 are given some of the distinguishing phenotypic characteristics that might be used for identifying the different geno-species proposed in Table 2.

According to the above proposal, the sub-polarly flagellated, slow-growing rhizobia, exemplified here by the two *R. japonicum* strains (see Table 1), should be incorporated into a separate new genus. Their relatively high percentage of GC, typical physiological features, and the results of numerical analysis (13) all support such a move. However, we feel that this important step should be backed up by hybridization experiments involving more strains. Temporarily, therefore, we prefer to keep the sub-polarly flagellated nodule-inducers in the genus as *R. japonicum*.

The group of bacteria producing nodules on the roots of *Leguminosae* and fixing free nitrogen during this symbiosis has been designated by four different generic epithets: *Phytomyxa* Schroeter, 1886; *Rhizobium* Frank, 1889; *Rhizobacterium*

Kirchner, 1896; and *Rhizomonas* Orla-Jensen, 1909 (4). The type species *R. leguminosarum* is the same for all of them. *Rhizomonas* Orla-Jensen, 1909 is already a *nomen generum rejiciendum* (4). According to the rules of the International Code of Nomenclature of Bacteria and Viruses, *Phytomyxa* Schroeter, 1886 should be used as the earliest and only legitimate name. However, only *Rhizobium* Frank, 1889 (val. pub., illeg.) has been internationally accepted and has come into common use. *Phytomyxa* and *Rhizobacterium* (illeg.) have been ignored and not employed.

To avoid disadvantageous changes in the nomenclature of this genus, by the strict application of the rules, we propose under provision 2 for Exception to The Rules: (i) that *Phytomyxa* Schroeter, 1886 be made a *nomen generum rejiciendum*; (ii) that *Rhizobium* Frank, 1889 [type species: *R. leguminosarum* (Frank) Frank, 1889] be made a *nomen generum conservandum*; and (iii) that *Rhizobacterium* Kirchner, 1896 be made a *nomen generum rejiciendum*. The opinion of the Judicial Commission of the International Committee on Bacteriological Nomenclature is requested.

DNA hybridization data to date indicate that the agrobacteria and rhizobia are genetically more related to the *Pseudomonadales* than to the *Eubacteriales*. Thus, in addition to changes at the genus and species level, a change in the higher classification of these bacteria may also be in order. If supergeneric classifications are to be maintained at all in the future, and if they are to have significant genetic or evolutionary meaning, it would seem prudent to wait for more data to accumulate before proposing any new groupings that may otherwise prove to have been made prematurely.

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