

## NOMENCLATURE OF THE ROOT-NODULE BACTERIA OF THE LEGUMINOSAE

I. L. BALDWIN AND E. B. FRED

*University of Wisconsin, Madison, Wisconsin*

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In Bergey's Manual of Determinative Bacteriology, second edition, 1925, the root-nodule bacteria of the Leguminosae are classified in the genus *Rhizobium*, and two species *Rh. leguminosarum* and *Rh. radicum* are recognized. Differentiation between the two species is established upon the basis of morphology, with particular reference to flagellation, cultural characters, and habitat of the organisms. Only incomplete descriptions of the organisms are given, which fact renders an accurate classification and identification difficult. With the exception of the habitat, no characteristic is described in sufficient detail to render identification possible, and only a very limited number of host plants are mentioned under each bacterial species.

In the seventh edition of Lehmann and Neumann's Bakteriologische Diagnostik, 1927, the nodule bacteria of the various leguminous plants are classified as one species, *Bacterium radicum* Beijerinck. The possibility of recognizing several subspecies or varieties is mentioned.

At present both American and foreign investigators use several different names for the same organism. Scientific workers have not agreed, either as to the number of species which should be recognized, or as to the terminology which should be applied to them. To avoid confusion many have abandoned the use of scientific terminology and have substituted such terms as "root-nodule bacteria of legumes" or, when a more specific designation was desired, such terms as "alfalfa-nodule bacteria," "soybean-nodule bacteria" or "alfalfa bacteria," "soybean bacteria." At

best this can be regarded only as a temporary expedient and not as a solution of the question.

Certain other investigators have used the scientific terminology, e.g., *Rhizobium leguminosarum*, *Rh. radicum*, *Bacillus radicum*, or *Pseudomonas radicum* without specifying the plant species from which the organism was secured. The inclusion of organisms differing as widely in their morphology and physiology as the root nodule organisms from alfalfa and bean in a single species *Rhizobium leguminosarum*, as is done by Bergey, leads to confusion. This is particularly true in such reports on the physiology of these microorganisms as that of Werkman, 1927.

The evidence in favor of the use of *Rhizobium* Frank as a generic term for this group seems quite conclusive to the writers. Since Buchanan (1926) has recently given an excellent review of the literature dealing with the synonymy of this group and has presented the facts regarding the use of this term, it is unnecessary to cover this material again, *Rhizobium* was included in the list of names recommended by the Committee of the Society of American Bacteriologists on Characterization and Classification for adoption by the Society; Bergey has adopted the term in his Manual of Determinative Bacteriology; and it has been commonly used by many scientists.

The genus *Rhizobium* was placed in the Family *Nitrobacteriaceae* and the Tribe *Azotobactereae* by the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacteria, and by Bergey in his Manual of Determinative Bacteriology. Löhnis and Hansen, 1921, and many others have criticized this classification of the root nodule bacteria, and have insisted that the organisms are closely related to the colon-aerogenes and the colon-typhoid groups. In the opinion of the authors the view of Löhnis is the more nearly correct one. Certainly the root nodule organisms of the Leguminosae are very similar to certain of the plant pathogens in their morphology, physiology and cultural characteristics: e.g., *Phytomonas tumefaciens*, of the family *Bacteriaceae* and the tribe *Erwineae*. Also, the genus *Rhizobium* has very little in common with the autotrophic bacteria of the family *Nitrobacteriaceae*.

The writers suggest that it would be advisable to transfer the genus *Rhizobium* from the *Nitrobacteriaceae* to the *Bacteriaceae* and place it in a new tribe standing close to the *Erwineae* and the *Bactereae*. *Hyphoidees* was suggested by Vuillemin, in 1905, since it drew attention to the "false hyphae" or "infection threads" formed in the nodule. Dangeard, 1926, used the term in his classification as a tribal designation. *Hyphoideae* would thus appear to be the proper designation for the tribe containing the genus *Rhizobium* and it is considered advisable to place this tribe in the family *Bacteriaceae*.

The early students in this field were for the most part of the opinion that all the organisms producing nodules on the roots of leguminous plants should be considered as a single species, although many investigators recognized that certain important differences were apparent among the representatives from the different plant species.

Frank, in 1879, noted certain differences between *Lupinus* and *Lathyrus* as to the type of nodule produced and the organisms contained in the nodule. He believed them all to belong to a single species and applied the name, *Schinzia leguminosarum*. Prior to this, 1877, Frank had stated that the fungus of the root nodules was *Schinzia cellulicola*.

Schroeter, in 1886, was the first to make a division of these organisms into two species. He transferred them from the genus *Schinzia* into a new genus *Phytomyxa*, and recognized two species: *Phytomyxa leguminosarum* (Frank) Schroeter and *Phytomyxa lupini* Schroeter. He said that the first species forms nodules on most leguminous plants, e.g., *Trifolium repens*, *Lotus corniculatus*, *Orobus vernus*, etc.; while the second forms nodules on *Lupinus luteus* and *L. angustifolius*.

Beijerinck, in 1890, reported that the differences between the organisms of the root nodules were greater than he had earlier supposed, and that *Bacillus Ornithopi*, from *Serradella*, should be recognized as distinctly different from *Bacillus Fabae*, from *Vicia faba*. This is apparently his first use of the species designation *Bacillus Fabae*. In earlier papers and also in the same article he referred to this organism as *Bacillus radicolica* var. *Fabae*.

In 1888, he had recognized that not all the organisms are identical, and had listed seven varieties of *Bacillus radicum*, namely varieties *Fabae*, *Vicia hirsutae*, *Trifoliorum*, *Pisi*, *Lathyri*, *Lupini*, and *Cytisi*, as well as mentioning *Phaseolus* and *Robinia*.

Schneider in 1892, described and named five species, with two varieties of one: *Rhizobium mutabile*, *Rh. curvum*, *Rh. Frankii*, varieties *majus* and *minus*, *Rh. dubium*, and *Rh. nodosum*.

Gonnermann, in 1894, secured cultures of several organisms from nodules of leguminous plants which were classified into two species with several varieties of each. Discarding all the earlier nomenclature, he named his organisms *Bacillus tuberigenus* and *Micrococcus tuberigenus*.

Kirchner, in 1895, studied the organism from soybean nodules and concluded that this organism is distinct from the others. The name *Rhizobacterium japonicum* was applied to it.

Many other cases might be cited in which the early scientific workers recognized that not all the nodule organisms of leguminous plants should be classed as belonging to a single species.

At the present time most workers agree that at least two species should be recognized, and a considerable number believe that sufficient differences exist to make necessary the creation of several species. As examples of the second group, recent papers by Dangeard, 1926, and Müller and Stapp, 1925, may be mentioned. Müller and Stapp, on the basis of the morphological, cultural, and physiological characters of the organisms, have separated eleven subgroups of the nodule bacteria of the Leguminosae. No scientific names have been used, nor species designations made. The authors hold, however, that it is possible definitely to identify each subgroup, and suggest that they should be classified on this basis. Dangeard, basing his work largely on careful studies of the nodules, decided that at least 10 species should be recognized. These are given names as follows: *Rhizobium Trifolii*, *Rh. polymorphum*, *Rh. Fabae*, *Rh. Meliloti*, *Rh. Loti*, *Rh. simplex*, *Rh. torulosum*, *Rh. Phaseoli*, *Rh. minimum*, *Rh. Sojae*.

Various workers have formed their classifications on the basis of either one, or a combination of more than one of the following

characters: (a) morphology; either on culture media or in the nodule; (b) cultural characters; type of colony and growth upon laboratory media; (c) physiology; as exhibited in the phenomena of plant inoculation, in the laboratory upon culture media, and in serological reactions. These characters are generally recognized at present as legitimate bases for species-differentiation.

Robson (1928), in his book entitled "The Species Problem" has brought out some worth-while suggestions regarding speciation. He considers that species in bacteria are probably of the same order as in higher animals. But they may well be less permanent from a human point of view and that with the admission of criteria other than morphological, pathogenicity becomes a more valid ground for specific differentiation.

Perhaps the most nearly constant of the physiological properties of the root-nodule bacteria is their ability to cause the formation of nodules upon certain species of the Leguminosae and not upon others. Certainly, from the practical standpoint, this is one of the most important characters of the organism. For more than twenty-five years, a utilitarian classification into "cross-inoculation" groups has been in use by students in this field. In the opinion of the writers, this characteristic is the most logical and valuable one on which to base any differentiation of the genus *Rhizobium* into species. The character which is now universally used to separate the members of this genus from other bacteria is the ability to form nodules on the roots of leguminous plants. If a further classification is to be made of the members of this genus, should not this character be used as a species identification? Detailed studies have shown, also, that the members of each "cross-inoculation" group differ from those of each of the others, in some or all of the following points: morphology, cultural characters, physiological properties, and serological reactions.

It may well be questioned whether the differences which are recognized are of a magnitude to justify specific or only varietal distinctions. To date no one in the field of bacteriology has been able to define clearly the characters upon which specific separa-

tions are to be based. As in all other fields of natural science, the judgment of the student, based upon, and in conformity with, similar cases, must be the deciding factor. In general, bacteriologists have agreed that a clear and definite difference in some essential character is a just and reasonable basis for species-separation. It is considered that the differences exhibited between the "cross-inoculation groups" are of that magnitude. Also further confusion must arise if the "cross-inoculation group" is to be recognized as a variety instead of a species, for it is clearly recognized that the members of the different "cross-inoculation groups" are further differentiated into subgroups based on cultural, physiological, and serological characters.

In view, therefore, of the fact that the root-nodule organisms naturally fall into several groups, each of which presents definite and constant characters differentiating it from all other organisms, and because of the confusion arising from the present system of classification, it is proposed that several separate species be established.

In the present proposal species-identification is based upon the morphology, cultural characters, physiological properties, and serological reactions of the bacteria. Many detailed reports have been published covering these characteristics, and only a few of the more outstanding points will be mentioned at this time. For more complete descriptions reference is made to the papers of Burrill and Hansen, 1917, Löhnis and Hansen, 1921, Wright, 1925, Stevens, 1925, Müller and Stapp, 1925, and Baldwin and Fred, 1927. Many others might be mentioned. In each case in which sufficient study has been made to render it possible, a species is established for each "cross-inoculation group." In deciding upon the nomenclature appropriate for these species, the authors have been guided by the rules of the International Code of Botanical Nomenclature.

1. *Rhizobium leguminosarum* Frank. The organism causing the formation of nodules upon the roots of *Lathyrus*, *Pisum*, *Vicia* and *Lens*.

a. Growth on mannitol agar is rapid with tendency to spread. Streak is raised, glistening, semi-translucent, and white. Con-

sistency is slimy and occasionally viscous. Considerable gum is formed.

b. Fermentation of carbohydrates. Slight acid-production from glucose, galactose, mannose, lactose and maltose.

c. Morphology. Peritrichous flagellation. Bacteroids from nodules are commonly irregular, with many X and Y shaped forms. Vacuolated forms predominate.

The species designation "*leguminosarum*" was proposed by Frank in 1879, when he erroneously placed the nodule forming organisms in the genus *Schinzia*. The greater portion of his work, as judged by his figures, was done with *Lathyrus* and *Orobus*, and *Orobus* is now placed in the genus *Lathyrus*. Frank recognized only the one species in the genus *Rhizobium*. If new species are to be established, the old species designation *leguminosarum* must be retained, and it would seem appropriate to apply it to the species including the organism causing the root nodules on *Lathyrus*.

2. *Rhizobium trifolii* Dangeard. The organisms causing the formation of nodules upon the roots of *Trifolium* sp.

a. Growth on mannitol agar is rapid. The colonies are white, becoming turbid as they grow older. Often the cultures become so mucilaginous that long threads may be drawn when the growth is touched with a needle. Streak cultures at first show a transparent growth along the line of inoculation. Later this growth becomes mucilaginous and flows down the inclined surface of the agar accumulating as a slimy mass at the bottom. Produces large amounts of gum.

b. Fermentation of carbohydrates. Slight acid—production from glucose, galactose, mannose, lactose and maltose. Usually slightly greater than with *Rh. leguminosarum*.

c. Morphology. Peritrichous flagellation. Bacteroids from nodules are pear-shaped, swollen, and vacuolated. Rarely X and Y shaped forms.

Beijerinck, in 1888, proposed *trifoliorum* as a varietal name for the organism isolated from *Trifolium*. Dangeard, in 1926, used the species term *trifolii*.

3. *Rhizobium phaseoli* Dangeard. Causes the formation of nodules on *Phaseolus vulgaris*, *Ph. angustifolia*, and *Ph. multiflorus*.

a. Growth on mannitol agar is rapid with tendency to spread. Streak is raised, glistening, semi-translucent, white. Consistency slimy and occasionally sticky but not so marked as in *Rh. trifolii*.

b. Fermentation of carbohydrates. Very slight acid-fermentation of glucose, galactose, mannose, sucrose and lactose.

c. Morphology. Peritrichous flagellation. Bacteroids from nodules are usually rods, with few branched forms. They are usually smaller than in *Rh. leguminosarum* and *Rh. trifolii*, and often vacuolated.

Beijerinck, in 1888, was able to distinguish the organisms of Phaseolus nodules from others and applied the term *Bacillus radicolica*, *phaseolus*-type. He did not, however, give it a definite varietal name. Schneider, in 1892, was the next to propose a specific designation for this organism. He proposed the term *Rh. Frankii* var. *majus* for the organisms symbiotic with *Phaseolus vulgaris*. *Rh. Frankii* var. *minus* was proposed for those symbiotic with *Pisum sativum*. Schneider's terminology is invalid from two standpoints. He failed to utilize any of the earlier specific and varietal names. His characterizations of the organisms are so meagre and conflicting that it is impossible to know definitely the organisms with which he worked. Dangeard, in 1926, used the term *Rh. phaseoli*.

4. *Rh. meliloti* Dangeard. The organism causing the formation of root nodules upon *Melilotus*, *Medicago*, and *Trigonella*.

a. Growth on mannitol agar is fairly rapid but not as fast as that of *Rh. leguminosarum*, *Rh. trifolii*, and *Rh. phaseoli*. Growth is moderate to abundant. The streak is raised, glistening, opaque, and pearly white. Consistency is buttery with considerable gum but usually not viscous.

b. Fermentation of carbohydrates. Strong acid-production from glucose, galactose, mannose and sucrose.

c. Morphology. Peritrichous flagellation. Bacteroids from nodules include both club-shaped and branched forms.

Dangeard first proposed this name in 1926, as applied to the organism symbiotic with members of the alfalfa-sweet clover



cross-inoculation group. Schneider in 1892 proposed the name *Rh. mutabile* for the organisms symbiotic with *Trifolium pratense*, *T. repens*, *Melilotus alba*, and *Lathyrus odoratus*. Schneider's terminology is invalid because of his failure to observe the rules of priority, and because of meagre and conflicting descriptions, rendering identification uncertain.

5. *Rh. japonicum* (Kirchner) comb. nov. The organism causing the formation of root nodules on *Soja max*.

a. Growth on mannitol agar is slow and scant under ordinary conditions. Streak is slightly raised, glistening, opaque, and white. Consistency is buttery with little gum formation. Pentose sugars give better growth than the hexoses.

b. Fermentation of carbohydrates. Little if any acid-formation. After prolonged incubation acid from xylose and arabinose.

c. Morphology. Monotrichous flagellation. Bacteroids of nodules are long, slender rods with only occasional branched and swollen forms.

This specific designation for the organism of the soybean root nodules was first proposed by Kirchner in 1895.

The five species described above comprise the list which is proposed at the present time. Specific designations for the organisms from the nodules of other leguminous plants are not proposed now, because of insufficient study and lack of definite information. As the study of these organisms continues, it will undoubtedly be desirable to increase the list of species.

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