NODULE BACTERIA OF LEGUMINOUS PLANTS

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INTRODUCTION

Despite the fact that the nodule bacteria of the leguminous plants have been made the subject of numerous publications, it is not to be disputed that their true morphological and physiological character, as well as their correct systematic position, are by no means sufficiently known. This is especially clearly demonstrated by the fact that they are still proclaimed by several writers to be the representatives of a special genus Rhizobium, once established by A. B. Frank as the result of rather inadequate studies upon this subject. In the new classification of bacteria, adopted by the Society of American Bacteriologists, the nodule bacteria again are widely separated from closely related species, and the error concerning the so-called genus Rhizobium has been revived once more.

Comparative investigations upon the symbiotic and the nonsymbiotic nitrogen-fixing bacteria of the soil, published in 1905 by the senior author, have proved conclusively that the nodule bacteria are not representatives of a special genus Rhizobium, but that they are closely related to *Bacillus radiobacter* Beijerinck and further to *B. lactis viscosum* Adametz, *B. pneumoniae* Friedländer, and *B. aerogenes* Escherich. The last three organisms are immotile, while the first one is motile; but here again the very close relationship between the immotile *B. aerogenes* and the motile *B. coli* has to be kept in mind. In fact, there can be easily isolated from every soil numerous varieties of *B. radiobacter*, which lead gradually up to *B. coli*, acquiring the power of fermentation and that type of growth on solid substrates which is characteristic of the last-named species. It has been pointed out in detail that all species mentioned above differ only gradually, not principally, in their physiological and morphological qualities, and especially that those branched or otherwise changed cell forms which are frequent in the root nodules are equally common with all members of this group of capsule bacteria, if these are tested adequately.[2] The ability to fix the atmospheric nitrogen was shown to be common in this group of organisms.

[1] Most of the experiments discussed in this paper were made in the summer of 1919, at the University of Illinois, where at that time the junior author held the position of Associate in Soil Biology. The photographs accompanying the paper were made by Mr. F. L. Goll, of the Bureau of Plant Industry, United States Department of Agriculture.

[2] It is not superfluous to emphasize once more that persistence in calling these forms "bacteroids" is by no means to be recommended. They are true bacteria, not foreign bodies looking like bacteria, as Frank's pupil Brunchorst erroneously believed. To speak of a "bacteroid" growth of bacteria is no less absurd than it would be to speak of a "fungoid" growth of fungi.

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Bacillus radiobacter was found to be peritrichic, and the same paper also indicated (*12, p. 592, footnote*)[1] that in all probability *B. radicicola* has the same kind of flagellation. But no faultless preparates were obtained at that time.

In the same year, 1905, G. T. Moore wrote concerning the nodule bacteria (*14, p. 26*):

There does not seem to be any necessity for creating a new group to include these organisms, as has been done by Frank, under the name of Rhizobium, for although there is a certain amount of polymorphism, it is no greater than frequently occurs in the bacteria.

With regard to the flagellation, however, Moore himself evidently made no special studies, and, accepting Beijerinck's statement that the "swarming bodies" (gonidia) of *Bacillus radicicola* are monotrichic as being valid for the bacteria too, he proposed to call the nodule bacteria *Pseudomonas radicicola.* Numerous authors have followed this suggestion, and experiments made by Harrison and Barlow (*8*) apparently confirmed the view that the flagellation of these organisms is indeed monotrichic.

However, these experiments are, in fact, not convincing, as has been emphasized especially by Kellerman (*9*). This author and also G. de Rossi (*16, 17*), Zipfel (*19*), and Prucha (*15*) secured results all of which demonstrated more or less clearly that the senior author's assumption was correct: *Bacillus radicicola* is peritrichic; it is no "Pseudomonas."

But this seemed again to be contradicted by certain results obtained by the junior author while working with the late T. J. Burrill (*6*). Numerous tests made with the bacteria isolated from cowpea, soybean, Japan clover, and other plants showed clearly and invariably monotrichic flagellation, and, therefore, the designation *Pseudomonas radicicola* was restored once more. Additional results, however, indicated that there are other features which differentiate the bacteria of the cowpea-soybean group from those living in the roots of clover, alfalfa, pea, and vetch. Especially the slime production and the speed of growth appeared to be different, and the organisms studied were arranged into two groups, "slow growers" and "fast growers" Both, however, were supposed to be merely varieties of *P. radicicola.*

This point remained to be investigated more thoroughly. In addition, another "fast grower" presented itself for detailed study, which quite regularly appeared on thickly sown plates of the "slow growing" groups, and which, indeed, has been mistaken by several investigators as the true nodule organism of cowpea, soybean, Japan clover, etc. Repeatedly such cultures were sent to and tested by the junior author. They were all unable to produce nodules.

The data given on the following pages make it evident that this "fast grower" is *Bacillus radiobacter*, which plays in this case, also, a very

[1] Reference is made by number (italic) to "Literature cited," p. 554–555.

interesting rôle. As this same species undoubtedly takes part in many processes occurring in soil and in water, it was thought useful to give another more detailed description of it, especially because, despite its ubiquity, *B. radiobacter* is much too little known. In addition to the rather short description given by Beijerinck, only the more complete one published by the senior author in 1905 exists thus far. On account of its great similarity to *B. radicicola*, *B. radiobacter* should be very well known to all bacteriologists working with the nodule bacteria in order to avoid mistakes which may otherwise not be discovered until only negative results are obtained in the inoculation tests.

Concerning the flagellation of the nodule bacteria three statements have been published more recently which also will have to be discussed presently. According to J. K. Wilson (*18*) the soybean bacteria are peritrichous; Barthel (*2*) declared lupine and alfalfa bacteria to be lophotrichous; Fred and Davenport (7) found the alfalfa organism peritrichous, but they found the lupine bacteria characterized by having one, rarely two, flagella.

EXPERIMENTAL RESULTS

The following strains of nodule bacteria were studied after having been tested with positive results in regard to their ability to produce nodules on the host plants from which they were isolated.

1. Cowpea.
2. Peanut.
3. Japan clover.
4. Beggar weed.
5. Soybean.
6. Red clover.
7. Sweet clover.
8. Vetch.
9. Strophostyles.

There were also included in our investigations two strains isolated from:

10. Black locust.
11. Lupine.

No positive inoculation test could be made on black locust. The lupine culture was kindly furnished by Dr. E. B. Fred, of the University of Wisconsin, who had tried it with positive results on this plant. Our tests were equally successful.

Two noninfectious "fast growing" cultures isolated from legume nodules and identified as *Bacillus radiobacter* were studied in comparison with six Radiobacter strains which originated from soil and which were kept in the senior author's collection since the years given in parentheses.

12. Fast grower from cowpea.
13. Fast grower from soybean.
14. *Bacillus radiobacter* from soil (1904).
15. Same (1907).
16. *Bacillus radiobacter* from soil (1908).
17. Same (1908).
18. Same (1908).
19. Same (1916).

No. 14 is the strain which in 1904 had been acknowledged by Prof. Beijerinck as being identical with his *Bacillus radiobacter* and which was used by the senior author for the original description published in 1905 (*12*).

TABLE I.—*Development of cowpea-soybean bacteria, Bacillus radicicola (from clover, vetch, etc.), and B. radiobacter*

Substrates.	Cowpea-soybean bacteria.
Mannite-nitrate agar slant.	MACROSCOPIC EXAMINATION.—Raised whitish to porcelain white, glossy layer. MICROSCOPIC EXAMINATION.—After 3 days slender rods, sometimes curved; after 7 to 10 days unstained, irregular sheaths, with 1 to 4, most frequently 2, darkly stained granules; after 2 to 3 weeks many small globules, ovals, and short rods outside of the unstained sheaths, also small globular regenerative bodies.
Beef agar slant.	MACROSCOPIC EXAMINATION.—Fairly good whitish growth. MICROSCOPIC EXAMINATION.—After 3 days weakly stained, irregular, thin, short rods; after 7 to 10 days irregular rods, producing gonidia and globular regenerative bodies, which may multiply as such; after 2 to 3 weeks very variable appearance, rather long slender rods, often branched, or club shaped, globular regenerative bodies, also unstained, irregular sheaths with dark granules, and large globular gonidangia.
Beef gelatin stab.	MACROSCOPIC EXAMINATION.—Very small, gray, nonliquefying disk on the surface, hardly any growth in the stab. MICROSCOPIC EXAMINATION.—Thin rods, sometimes branched or swollen, producing gonidia and small globular regenerative bodies; in old cultures gonidia and regenerative bodies frequently predominating.
Beef broth.	MACROSCOPIC EXAMINATION.—Broth at first clear, with little sediment; later (after about 2 weeks) slightly turbid. MICROSCOPIC EXAMINATION.—After 3 days slender rods, sometimes curved; after 2 weeks granular rods producing gonidia, also budding and branching, small globular regenerative bodies, and symplasm; after 3 to 4 weeks very irregular forms, branching, swelling.
Milk.	MACROSCOPIC EXAMINATION.—During the first weeks no change visible, later slow digestion, no clear serum zone. MICROSCOPIC EXAMINATION.—Mostly small globules and ovals, few short, slender rods.
Potato.	MACROSCOPIC EXAMINATION.—Very meager, transparent, thin layer. MICROSCOPIC EXAMINATION.—After 7 days slender rods, sometimes branched, or with terminal swelling; after 4 weeks small globules and ovals, irregular rods (frequently branched), globular regenerative bodies, and symplasm with very variable new development.

TABLE I.—*Development of cowpea-soybean bacteria, Bacillus radicicola (from clover, vetch, etc.), and B. radiobacter*—Continued

Substrates.	*B. radicicola* (from clover, vetch, etc.).	*B. radiobacter*.
Mannite-nitrate agar slant.	MACROSCOPIC EXAMINATION.—Slimy, transparent growth, with or without whitish streaks. MICROSCOPIC EXAMINATION.—Small ovals and short rods, producing after 1 to 2 weeks gonidia and small globular regenerative bodies. Also unstained slime threads with dark granules and large globular, or oval gonidangia; irregular pale forms from symplasm.	MACROSCOPIC EXAMINATION.—Thick, slimy transparent layer, with whitish streaks. MICROSCOPIC EXAMINATIONS.—After 7 days small ovals and short rods, imbedded in slime; after 14 days some rods with thick unstained capsules forming symplasm; after 3 to 4 weeks normal cells, stars, and large globules and clubs from symplasm.
Beef agar slant.	MACROSCOPIC EXAMINATION.—Meager, flat, grayish growth. MICROSCOPIC EXAMINATION.—Mostly small ovals and short rods, the latter sometimes curved, budding and branching; later also large rods, and large globular, oval, or club-shaped gonidangia.	MACROSCOPIC EXAMINATION.—Flat, whitish slimy layer, thick sediment below. MICROSCOPIC EXAMINATION.—As on mannite-nitrate agar.
Beef gelatin stab.	MACROSCOPIC EXAMINATION.—Small, gray, nonliquefying disk on surface, very little growth in stab. MICROSCOPIC EXAMINATION.—Small ovals and short rods, gonidia, and small globular regenerative bodies.	MACROSCOPIC EXAMINATION.—Grayish, flat, round, nonliquefying surface growth, little growth in stab; after 2 to 4 weeks gelatine sometimes brown on top. MICROSCOPIC EXAMINATION.—Typical ovals and short rods, these sometimes curved or branched, also unstained slime threads with dark granules, later symplasm with stars.
Beef broth.	MACROSCOPIC EXAMINATION.—Broth either clear or very slightly turbid, little whitish sediment. MICROSCOPIC EXAMINATION.—Small ovals and short rods, budding and branching, occasionally threads; after 1 to 2 weeks many gonidia and small, globular regenerative bodies.	MACROSCOPIC EXAMINATION.—Broth turbid, white ring, whitish film, much whitish sediment. MICROSCOPIC EXAMINATION.—Small ovals and short rods, budding and branching; later gonidia, globular regenerative bodies, threads, and fine stars from symplasm.
Milk.	MACROSCOPIC EXAMINATION.—After 1 to 4 weeks clear serum zone on top, 2 to 5 mm. thick; milk below nearly unchanged, very fine flocculation. MICROSCOPIC EXAMINATION.—Small ovals and rods, later also granular threads and symplasm.	MACROSCOPIC EXAMINATION.—First slime ring and serum zone on top; later whole milk turning brown. MICROSCOPIC EXAMINATION,—After 7 days typical ovals and rods; later small and large cells from symplasm.
Potato.	MACROSCOPIC EXAMINATION.—Meager, transparent, slimy growth. MICROSCOPIC EXAMINATION.—Small slender rods, budding and branching, some ovals, globular regenerative bodies; later gonidia predominant.	MACROSCOPIC EXAMINATION.—First gray, later coli-brown slimy layer, potato turns frequently gray. MICROSCOPIC EXAMINATION.—First small ovals and short rods, budding and branching, later also large oval and globular gonidangia and symplasm with stars.

The results of our investigations leave no doubt that the earlier findings of the junior author were correct so far as the polar flagellation and the peculiar morphological and cultural features of the cowpea-soybean organisms are concerned. On the other hand, it could now be ascertained with equal certainty that the bacteria producing nodules on clover, alfalfa, vetch, and other plants originally cultivated in Europe are all peritrichic and exhibit all the characteristics of *Bacillus radicicola*, as described by Beijerinck and other authors.

Those findings which were obtained most frequently and which may be considered as being typical for the two groups of nodule bacteria are compiled in Table I, together with analogous data pertaining to *Bacillus radiobacter*. Photographs of the most characteristic details are reproduced on Plates 68 and 69.

When grown from the root nodule on Harrison and Barlow's ash agar, mannite agar, or similar agar of low nitrogen content, the two groups of nodule bacteria are best characterized and differentiated by the very slow growth of colonies in the cowpea-soybean group and the comparatively quick growth of those of *Bacillus radicicola* (6, *pl. 11, fig. 1-11*). Frequently, but not always, the development of *B. radiobacter* is still somewhat more rapid than that of *B. radicicola;* in the macroscopical as well as in the microscopical aspects, however, the colonies of these two species on such media are so very much alike that it is almost impossible to distinguish them with certainty. Both, when developing on the surface, are perfectly round, drop-like, soft, watery or slimy, glistening, transparent. Often a whitish center or whitish streaks become visible within the more transparent mass, especially if the surface colony is the outgrowth of an imbedded colony. Sometimes it appears as if this whitish center were regularly to be seen only with certain strains of Radicicola and Radiobacter. This is not the case, however. Its presence or absence is erratic and can not be used for differentiation. The imbedded colonies are always small, white, opaque, mostly lentiform, less frequently circular. Under the microscope the surface colonies present themselves as sharp-edged disks, pure white at the outside with yellowish-grayish granulation in the center. In a few cases a radiate structure becomes visible. The colonies of the cowpea-soybean group appear macroscopically, as well as microscopically like young colonies of the Radicicola type. The presence of Radiobacter colonies on the plate stimulates their growth markedly.

In cell morphology there is again a more pronounced relationship between Radiobacter and Radicicola than between the nodule bacteria of the clover-vetch group on the one side and of the cowpea-soybean group on the other. This holds true with the regular rod forms as well as with the very pleomorphic, curved, swollen, branched, or otherwise changed types of growth characteristic of these groups. The photographs on Plate 68, D–L, represent the pictures we have seen most frequently, but they do not pretend to give a complete illustration of the wide pleomorphism of these organisms. Before their complete life history can be given much additional material will have to be collected, especially with regard to the form of gonidangia, regenerative bodies, and the various cells developing from the symplastic stage. At present we intend only to bring out as clearly as possible those points which are important for a correct differentiation and diagnosis. As far as one may judge from the microscopic appearance, it is the inclination of *Bacillus radiobacter* to form stars which is most characteristic (Pl. 68, L), and this was used, therefore, by Beijerinck for its denomination. With *B. radicicola* the frequent occurrence of the clear-cut, compact Y forms is the most conspicuous feature (Pl. 68, H); whereas the bacteria of the cowpea-soybean group present themselves in most cases, when stained with aqueous aniline dyes in the usual manner, as short or long, unstained sheaths with one or more darkly stained granules (Pl. 68, J). Of course Y forms, as well as unstained sheaths with darkly stained gonidia, can be observed not infrequently with the other organisms, too, and the star formation is by no means solely to be found with Radiobacter; but we feel sure that those pictures, as shown on Plate 68, G–L, will be found most valuable for diagnostical purposes.

The flagellation is the same with Radiobacter (Pl. 68, C) and Radicicola (Pl. 68, B), while the bacteria of the cowpea-soybean group are characterized by one coarse, fairly straight polar flagellum (Pl. 68, A). Just before fission one cilium may be seen at each end; as a rare exception a tuft of polar flagella was observed occasionally. Frequently a darkly stained body becomes visible within the rod just at that point where the flagellum springs forth, which may be considered to be a flagellated, not yet liberated, gonidium, such as can be seen occasionally with many other bacteria, especially with *Bacillus radicicola*, too. When liberated this becomes the monotrichic small "swarming body" described by Beijerinck in 1888 (*4*).

The growth on mannite-nitrate agar, as well as on beef agar slants, as described in Table I, is quite characteristic, and after the eyes have been sufficiently trained, one seldom makes a mistake in guessing the group to which a culture presented for inspection may belong. But it must be admitted that occasionally and temporarily a strain of the cowpea-soybean group can show the flat, transparent growth characteristic of Radicicola, whereas it is a very rare occurrence that a member of the last-named group simulates the former one. The growth of Radiobacter is always very typical, except when a very weak strain is encountered, which does not frequently occur within this group. Plate 69, A, demonstrates the characteristic differences noticeable on mannite-nitrate agar as clearly as they can be shown in a photographic reproduction.[1]

[1] As was the case with Azotobacter, for which the mannite-nitrate agar was first used (*13, p. 686*), so also the nodule bacteria and *Bacillus radiobacter* grew very readily on this substrate. Allen (*1, p. 33*) asserted recently that he could not get any growth of Azotobacter on a dextrose agar, which he erroneously called "Löhnis and Smith's medium." But not even the formula used by us has been quoted correctly by Allen, and it is, of course, quite obvious that on account of the alterations made by Allen his agar must indeed have been quite unsuitable.

Cultures on beef gelatine and in beef broth differentiate clearly Radiobacter and nodule bacteria, while, as stated in Table I, the two groups of nodule organisms grow very much alike on these substrates. Microscopic tests, however, made from gelatine and broth furnish, in most cases, especially characteristic pictures, provided that the growth has not been altogether too poor to get a satisfactory preparate.

The growth in milk and on potato, as described in Table I and illustrated on Plate 69, is very characteristic and can be used to great advantage for diagnosis. It is not to be denied that with old stock cultures atypical results may sometimes be obtained in this direction also. Especially cultures rich in or entirely made up of the globular regenerative bodies, which are produced by these as well as by all other bacteria, furnish whitish, yellowish, or only slightly brownish growth on potato in the case of *Bacillus radiobacter* and *B. radicicola*. But we have never seen such atypical growth with new isolations. Here the coli-brown color of the potato cultures separates Radiobacter sharply from the nodule bacteria, and these in turn are equally sharply to be distinguished by the behavior of their milk cultures. It is true that sometimes milk cultures of the *B. radicocola* group also leave the milk unchanged, but the microscopic test of such abnormal cases probably will always show, as it did in the cases studied by us, that the abnormality was simply caused by the fact that the bacteria which were inoculated did not multiply at all. Furthermore, no alteration may be seen if milk is used which has been kept for a long time and has become concentrated by evaporation of part of its water.

To determine on a larger scale whether this different behavior of the two groups of nodule bacteria, when grown in milk, can be correctly accepted as of real diagnostic value, all cultures of nodule bacteria at our disposal were tested simultaneously with the following results:

MILK WAS CHANGED AS TYPICAL FOR BACILLUS RADICICOLA BY THE FOLLOWING CULTURES:	MILK WAS LEFT UNCHANGED BY THE FOLLOWING CULTURES:
5 from red clover.	10 from cowpea.
4 from sweet clover.	8 from soybean.
6 from navy bean.	5 from peanut.
1 from vetch.	4 from Japan clover.
2 from lupine.	2 from beggar weed.
3 from black locust.	2 from *Cassia chamaecrista*.
3 from Amorpha.	
2 from Strophostyles.	

If kept for longer than four weeks milk cultures of the cowpea-soybean organisms usually become more or less transparent on account of partial decomposition of the casein; but they never show the perfectly clear zone characteristic of the other group.

The bacteria were also tested on other media besides the standard substrates, of which sterilized soil, moistened with 0.5 per cent mannite solution, mannite-nitrate solution as used for studying the life cycle of Azotobacter, tap water plus 0.5 per cent beef broth, and 2 per cent salt agar furnished the most satisfactory results, especially with regard to a more complete knowledge of the cell morphology of the organisms. For diagnostic purposes, however, these substrates are of minor importance, as they do not bring out anything which is not already to be seen on the standard media. Nevertheless, it should be pointed out that cultures of the nodule bacteria in soil are to be recommended for two reasons. First, they are useful in keeping the organisms in a normal state of virility for a long time, and, in the second place, they demonstrate very clearly, when studied microscopically, that it is erroneous to believe—though numerous authors have promoted such hypotheses—that the nodule bacteria behave very differently in soil and could, therefore, not be isolated in their typical form from their natural habitat. Our results are in complete agreement with those recently obtained by Barthel (*3*) concerning the growth of bacteria in sterilized soil.

Tap water containing 0.5 per cent beef broth gave also very good development and proved repeatedly helpful in reviving old, weakened strains which refused to grow on solid substrates.

DISCUSSION

Our experimental results leave no doubt that the nodule bacteria of the leguminous plants are to be divided at least into two distinct groups, differing morphologically as well as culturally. It is equally beyond dispute that these differences are so marked and constant that one might be inclined to establish the nodule organism of the cowpea-soybean group as a new species. On account of its behavior in the inoculation test O. Kirchner has considered the soybean organism a distinct species, which he named in 1895 *Rhizobacterium japonicum*(*10*). According to the rules of priority, this species name would have to be given preference, despite the fact that different behavior in the inoculation test generally can not be accepted as a distinguishing mark of such quality as to validate the creation of a new species. The generic name Rhizobacterium, used by Kirchner, is, of course, equally as untenable as the generic name Rhizobium. According to the two tmost requently used modes of classifying the bacteria, one might name the cephalotrichic non-sporulating rod of the cowpea-soybean group *Pseudomonas japonica* or *Bacterium japonicum*, while the name *Bacterium* or *Bacillus radicicola* would have to be retained for the peritrichic organisms to be found with clover, alfalfa, sweet clover, vetch, pea, etc.

However, we do not advocate such a procedure. We are firmly of the opinion that much more must be known of the complete life history of a bacterium than is obtainable along the standardized lines of customary bacteriological research, before one can safely proceed to establish a genuine species on a truly scientific basis. Undoubtedly many if not most of the commonly used so-called species names of bacteria are no species names at all, but merely denominations more or less correctly applied to organisms about whose complete life history and, accordingly, about whose true systematic value and position comparatively little is known at present.

It is by no means impossible that future systematic investigations may demonstrate the peritrichic and the cephalotrichic nodule bacteria to be relatively constant types of growth of one species. There are a few reports in the literature indicating that occasionally cross inoculations have been obtained, which might support this hypothesis. While O. Kirchner never found nodules on soybeans grown in Germany and therefore thought his *Rhizobacterium japonicum* to be the active agent in the Far East, F. Cohn said in a note appended to Kirchner's report that soybeans grown for the first time in his experimental garden in Breslau did produce nodules, though these were not of the normal type and contained only a few rodlike bacteria. Kellerman reported upon a case where a culture originally isolated from alfalfa was found to be infective on alfalfa and lupine as well as on soja when tested by Leonard after six years' cultivation on artificial substrates. It may be mentioned also in this respect that cross inoculations between navy bean and cowpea seem to be equally possible, under circumstances, however, which need further elucidation.

But just as negative results in cross inoculation experiments can not be accepted as sufficient basis for establishing different species, so also such rather exceptional positive results can not be used as valid support of the hypothesis that monotrichic and peritrichic nodule bacteria are only two types of growth of the same species. First of all, it would have to be ascertained whether in such cases the peritrichic organism has really changed into the monotrichic one, or vice versa. The possibility remains, of course, that occasionally the one type of organisms may invade a host plant whose nodules are normally caused by the other type of bacteria.

Changes in flagellation from peritrichic to cephalotrichic have been observed, according to Lehmann and Neumann (*11, p. 256, 357, 371*), with *Bacillus coli* and *B. alcaligenes*. Both species are related to *B. radiobacter* and *B. radicicola*, and under this aspect an analogous change should not be rejected prematurely as *a priori* improbable.

At the end of the introduction three statements have been quoted from the more recent literature which one might be inclined to accept as confirmative evidence in this direction. However, on account of the following reasons we do not feel justified in advocating such an interpretation.

J. K. Wilson says that in his preparations of soybean organism—

The flagella were peritrichous, the highest number found being four.

As no photomicrographs had been made, Dr. Wilson was kind enough to furnish, on request of the senior author, one of his slides for examination. The flagella visible therein were all very weakly stained, so that no definite conclusion could be drawn. A culture, for which we are also indebted to Dr. Wilson, behaved in our hands like all those tested before; practically all cells were distinctly monotrichous. A comparison of Plate 68, A, with the pictures published on Plates IV and V of Bulletin 202, Illinois Agricultural Experiment Station (*6*), leaves no doubt about this point.

In Barthel's paper (*2, p. 16*) two drawings and one photomicrograph are to be found which clearly illustrate the following statement:

> Bei den Lupinenbakterien sind die Geisseln ziemlich lang, wellig geformt und an einem Pole befestigt. Ihre Anzahl variiert von 1 bis 6. Ihre Placierung ist recht eigentümlich. Sie sitzen nämlich öfters nicht gerade an der Spitze des Zellleibes, sondern sozusagen an den "Ecken" und oft etwas von dem Hinterende entfernt. Oft findet man auch eine Geissel an der einen "Hinterecke" und mehrere andere zusammen an der anderen. . .
>
> Bei den Luzernebakterien waren die Geisseln meist weniger und kürzer, am häufigsten 1 oder, seltener 3 oder 4, aber auch hier deutlich lophotrich. . .

Fred and Davenport (7), on the other hand, saw only one or two cilia with the lupine bacteria, while several strains of alfalfa organisms left no doubt as to their peritrichic flagellation.

We believe that these conflicting views are in fact not so irreconcilable as they seem to be. If well-made slides are examined carefully, some cells will always be discovered which clearly show that on account of the primary swelling and the following shrinking of their capsules, the flagella are often more or less dislocated. Some of the cells shown in Plate 68, A–C, exhibit this phase as clearly as it is possible in such reproductions. The flagella of the monotrichous bacteria of the cowpea-soybean group are to be seen in an exactly polar position only when the cells themselves are lying lengthwise within the "drift," as indicated by the floating flagella. In all other cases dislocations may take place, removing the cilia to the corners or even to the side of the cells, where they should not be viewed, however, as remnants of a peritrichic flagellation.

On the other hand, analogous disturbances may cause the occurrence of apparently cephalotrichic bacteria among the peritrichic cells of *Bacillus radicicola* and *B. radiobacter*. That there exists no truly polar flagellation in these cases, however, is evidenced by the fact that the cilia composing such an apparently polar tuft do not protrude exactly from the same spot, as they do, for example, in the cell with several polar flagella shown in Plate 68, A. They are always more or less separated and are only accidentally drawn together in the course of the shrinking of the capsule. A thorough examination of well-made preparations leaves no doubt that the original position of the flagella is peritrichic.

SUMMARY

(1) The nodule bacteria of the leguminous plants are to be divided into two groups, differing morphologically as well as physiologically.

(2) The first group shows all features characteristic of *Bacillus radicicola* Beijerinck. It is peritrichic, grows relatively fast on agar plates, and changes the milk in a very characteristic manner. It produces nodules on the roots of the following plants: clover, sweet clover, alfalfa, vetch, pea, navy bean, lupine, black locust, Amorpha, and Strophostyles.

(3) The second group is characterized by monotrichic flagellation, comparatively very slow growth on agar plates, and inability to cause a marked change in milk. It has been isolated from cowpea, soybean, peanut, beggarweed, Acacia, Genista, and Cassia.

(4) According to the customary manner of classifying bacteria, this second group of nodule bacteria would have to be considered to be a new species, and according to the rules of priority, it would have to be named *Pseudomonas japonica* or *Bacterium japonicum* (Kirchner). But we do not advocate such a procedure, because only a complete study of the life history of these two groups of organisms would make it possible to say definitely whether they are, indeed, two distinct species or merely different types of growth of the same organism.

(5) *Bacillus radicicola* is closely related to *B. radiobacter*. The generic name Rhizobium is to be rejected. The correct systematic position of both species is in the neighborhood of *B. aerogenes* and *B. coli*.

(6) *Bacillus radiobacter* seems to be regularly present in the root nodules of leguminous plants, stimulating development and activity of the nodule bacteria. On account of its similarity to *B. radicicola*, it has been repeatedly mistaken for the nodule-producing organism in the cowpea-soybean group, whose bacteria it outranks very considerably in the development on the plates made from the nodules. By its brown growth on potato, *B. radiobacter* can be easily differentiated from *B. radicicola*.

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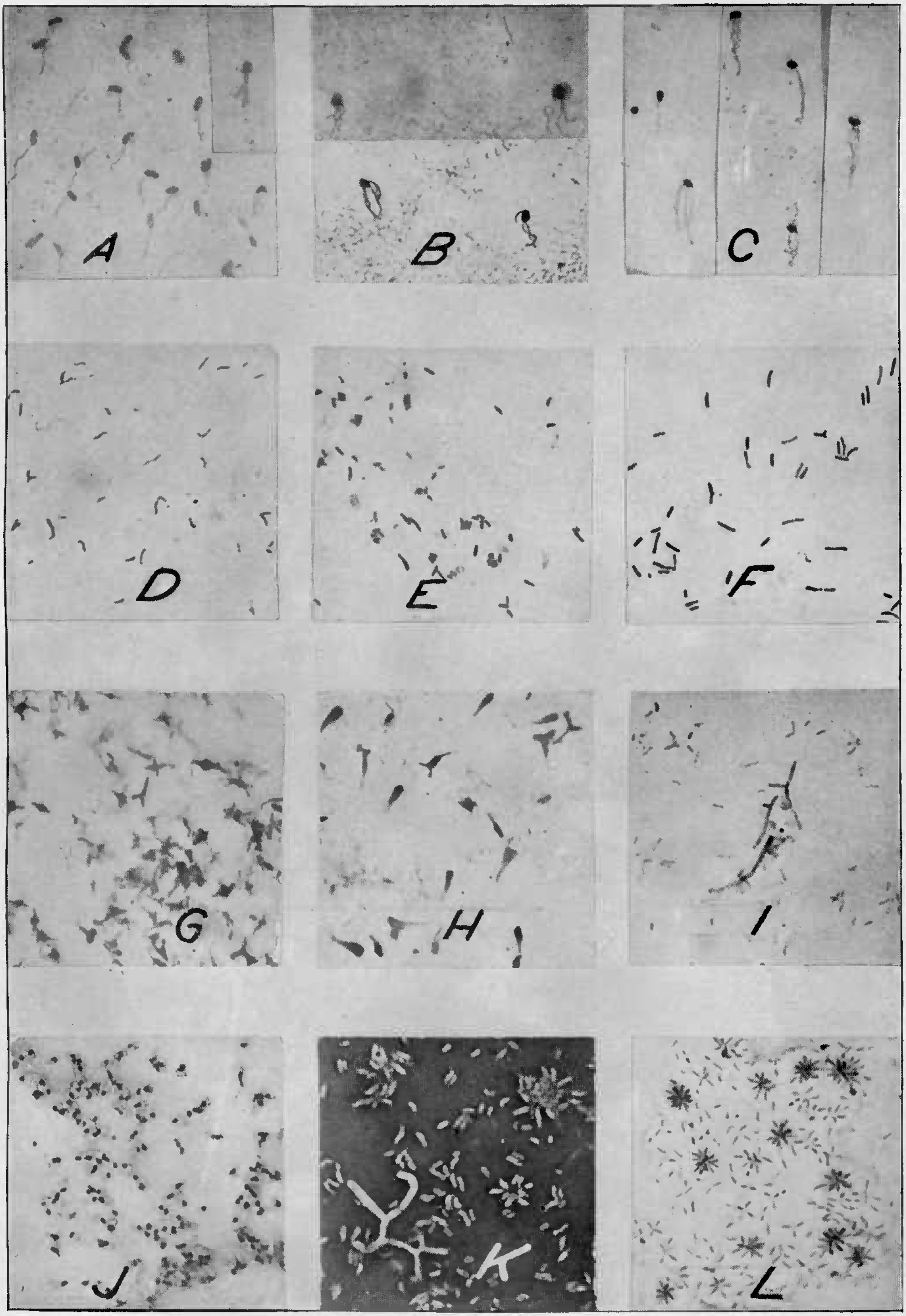
PLATE 68

A.—Soybean bacteria, J. K. Wilson's strain, 4 days old.
B.—Vetch bacteria, 3 days old.
C.—Bacillus radiobacter, 2 days old.
D.—Soybean bacteria, beef agar, 4 days old.
E.—Red clover bacteria, beef agar, 4 days old.
F.—*Bacillus radiobacter*, beef agar, 4 days old.
G.—Cowpea bacteria, potato, 6 days old.
H.—Red clover bacteria, potato, 14 days old.
I.—*B. radiobacter*, milk, 7 days old.
J.—Cowpea bacteria, mannite-nitrate agar, 8 days old.
K.—Vetch bacteria, mannite-nitrate agar, 8 days old.
L.—*B. radiobacter*, mannite-nitrate solution, 17 days old.

A–C Loeffler's stain; D–L aqueous fuchsin. ×1,000.


A
B
C
D
E
F
G
H
I
J
K
L

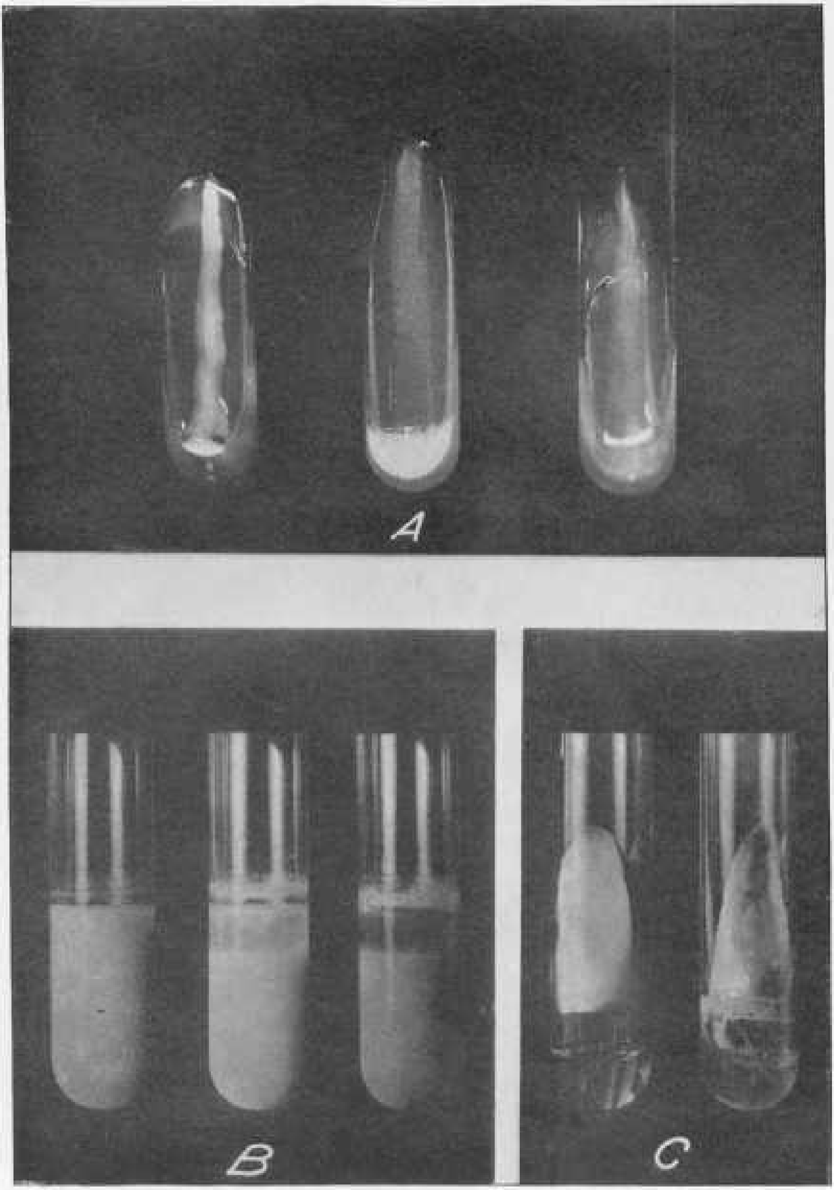

A
B
C

PLATE 69

A.—Mannite-nitrate agar slants, 8 days old, from left to right: soybean bacteria, vetch bacteria, and *Bacillus radiobacter*.

B.—Growth in milk, 4 weeks old from left to right: soybean bacteria, vetch bacteria, and *B. radiobacter*.

C.—Growth on potato, 2 weeks old: vetch bacteria (left) and *B. radiobacter* (right).