

TAXONOMIC RELATIONSHIPS OF ALCALIGENES SPP.
TO CERTAIN SOIL SAPROPHYTES AND PLANT
PARASITES¹

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Recently one of the writers (Conn, 1938) proposed the name *Rhizobiaceae* for a family containing the genera *Rhizobium*, *Alcaligenes*, and *Chromobacterium*, together with certain species commonly placed today in the genus *Phytomonas*. This suggestion has been followed in the 5th edition of Bergey's Manual of Determinative Bacteriology. Inasmuch as the writer's paper above mentioned was published only in abstract form, it seems wise to present in greater detail the data on the basis of which the suggestion was made.

Some 35 years ago (Conn, 1913), interest was expressed in a group of non-spore-forming rods, which are very abundant in soil, which are usually gram-negative and slow liquefiers of gelatin. Various efforts have been made to classify the group and to decide to what other groups of bacteria they are related. This proved difficult because these organisms are characterized so largely by negative features. A name was given (Conn, 1928) to one organism of this group, however, (*Bacterium globiforme*) which is easily recognized because in its life history on agar slant cultures it changes from a gram-negative rod to a gram-positive coccus.

A little later it was recognized that there is a similar group of bacteria present in milk which are ordinarily called alkali-formers because the only change produced in litmus milk is a slight alkalinity. Best known among this group of bacteria is the cause ofropy milk, commonly named until recently *Bacterium viscosum*.

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Still more recently it was realized that a common soil saprophyte, *Bacterium radiobacter*, (Beijerinck & Van Delden) Löhnis, commonly found associated with the roots of legumes, has similar physiological characteristics. This organism, so similar to the legume nodule bacteria (*Rhizobium spp.*), is often confused with species thereof. The species included in the genus *Rhizobium*, in turn, are almost indistinguishable culturally from one or two of the plant pathogens, especially *Phytopomonas tumefaciens* (Smith & Townsend) Bergey, *et al.*

The organisms just referred to differ so widely in habitat that their similarities have only recently been appreciated; and in early editions of Bergey's Manual they were placed in widely separated genera. The artificiality of their classification became evident if one tried to identify one of them by means of the keys in the 4th edition of Bergey's Manual for one would arrive at the genus *Achromobacter* or *Alcaligenes* according to whether or not one disregarded pathogenicity as a valid point for the separation of groups of genera. *Alcaligenes* was placed in the group of pathogens, *Achromobacter* among the saprophytes. In spite of this fact, six out of the ten species of *Alcaligenes* listed were saprophytic. In other words, there seems to be no recognizable distinction between *Alcaligenes* and the similar organisms which were then placed in *Achromobacter*.

This similarity between *Alcaligenes* and some of the soil and milk organisms made it seem well to undertake a study of the genus, and especially of its type species. If its type, *Alcaligenes faecalis* Castellani and Chalmers (syn. *Bacillus faecalis alcaligenes* Petruschky), is recognizable and does not differ too greatly from the organisms here under discussion, this genus seems to offer a good place to put some of the latter. As a matter of fact, the 3rd and 4th editions of Bergey did recognize the rosy milk organism under the name *Alcaligenes viscosus*.

The original idea of the similarity of these various organisms was obtained purely because of their inability to produce appreciable acid from sugars. When it was found later that many of them tend to have a definite type of flagellation, the logic of placing them together seemed inescapable. The type of flagel-

lation in question is that described by two of the writers (Conn and Wolfe, 1938a) as degenerate peritrichous flagellation. It was first encountered in the case of a legume nodule organism, which was sometimes called peritrichous and sometimes monotrichous, Burrill and Hansen (1917) showing that if only one flagellum was present it was as apt to be at the side as at the pole. Hofer (unpublished work) found that *Bacterium radiobacter* has the same type of flagellation. The violet chromogenic bacteria (*Chromobacterium*) have also been described sometimes as peritrichous and sometimes as monotrichous.

The writers now present evidence that this is a common type of flagellation among soil bacteria and that it is also found in *Alcaligenes faecalis* as well as in these groups mentioned above which have sometimes been described as having several, and sometimes only one, flagellum.

WHAT ARE THE LIMITS OF THE RHIZOBIACEAE?

Granted that there is a natural group of bacteria to which the family name *Rhizobiaceae* may be assigned, the question arises as to what organisms should be included in the group. The organisms discussed above are motile, prevailingly gram-negative, and seem to have the common ability of converting sugar, under favorable conditions, almost wholly into carbon dioxide and cell substance, without the production of organic acid. Certain *non-motile*, *non-spore-forming* bacteria, however, with all the common characteristics of the above except possession of flagella, occur abundantly in soil. They are often considered together with the motile bacteria discussed because they apparently belong to a rough grouping of organisms called by Winogradsky (1924) the autochthonous flora of soil. He gave this name to certain types which were minute rod-shaped or coccoid forms that seemed to be indigenous to soil and to maintain continual high numbers without much fluctuation regardless of what nutrient materials were incorporated with the soil. The senior author (1935) showed that organisms having the morphology described by Winogradsky and occurring in soil under the conditions observed by him, have the type of physiology here under discussion.

This makes it of interest to consider the motile and non-motile organisms together and to study their relationships to one another, even though they may be shown to belong to quite divergent groups.

The classification of these last mentioned organisms has been a puzzle for some time. Lochhead and Taylor (1938) summarize the situation by stating that these bacteria are "physiologically unstable" and hence cannot be classified by biochemical means. Whether this view is correct or not, it is certain that no progress has been made in efforts to group them on the basis of the conventional biochemical tests commonly applied to bacteria. Of these autochthonous soil types the only organism to which one of the writers has attempted to assign a specific name is *Bacterium globiforme*, which can be recognized by its tendency to change from a gram-negative rod to a gram-positive coccus when about two days old, on agar slant culture.

In the 4th edition of Bergey's Manual this species was placed in the genus *Achromobacter*; but in the 5th edition it has been returned to its original position in *Bacterium*. This latter genus is retained in the current edition for those species whose relationships are still uncertain. This is an illustration of the difficulty in classifying an organism showing such a type of morphology.

It has been suggested that *Bacterium globiforme* may be related to those soil organisms which show filamentous, club-shaped, or branching forms in the course of their life cycles. Some authors (Topping, 1938) conclude that these belong in the genus *Mycobacterium*, whose type species is the tubercle organism. Jensen (1934) described numerous species from soil which he has placed in this genus. At first thought it would seem that these soil forms are quite unlike the type species of *Mycobacterium* in physiology; but although the tubercle bacillus is a pathogen, it will grow in some substrata not unlike the synthetic media in which the soil bacteria thrive. Therefore cultures labelled *Mycobacterium* and *Corynebacterium* were obtained from Jensen to compare with those studied in this work.

It has been found that physiologically the latter organisms are very similar to others referred to in this paper. Morphologic-

ally, however, they seem to be fairly distinct because of their tendency to show true or false branching. The most extreme organisms of this type are some obtained from Jensen which sometimes show a chalky appearance of the growth on agar similar to that obtained by *Actinomycetes*. Standing next to these seem to be the gram-positive organisms which show branching cells in the little plate technic described by Topping (1938) but do not appear chalky. Next should be placed the gram-positive organisms (which Jensen called *Corynebacterium*) which do not show true branching but have the so-called "snapping" of the cells after division which sometimes brings about false branching. Next to this might be placed the *Bacterium globiforme* type which changes from gram-negative rods to gram-positive cocci and which occasionally shows branched or club-shaped cells. Then, finally, come the gram-negative non-motile rods which show no tendency to produce irregular-shaped cells. These, in their turn, differ from the motile organisms we have been considering only in their lack of flagella.

In other words, a series of organisms have the type of physiology here under discussion, the extremes of which differ so widely in morphology that one can hardly consider grouping them together. The non-motile forms of this series deserve further study. This problem is not considered in the present paper. The reason for discussing them here is because of the difficulty in drawing the line between them and the typically motile forms of the *Rhizobiaceae*. Since the latter include vigorously motile forms with 3 or 4 flagella and sluggishly motile organisms with one, it is hard to exclude from them non-motile rods of similar size, shape, and physiology. Motility is a character that is easily lost.

If the division is not based on motility, the most obvious point of distinction seems to be between forms that are prevalingly gram-positive and those that are predominately gram-negative. The *Rhizobiaceae* are typically negative. The *Mycobacteriaceae* are typically positive. The only difficulty in this regard is in the case of *Bacterium globiforme* which undergoes a change in morphology and a coincident change in gram reaction. This or-

ganism must therefore for the present be regarded as an intermediate form between the two families under consideration; hence it seems best to keep it, until further study has been made, in the indefinite genus *Bacterium*.

The definition of the family *Rhizobiaceae* as given in the 5th edition of Bergey's Manual is as follows:

Cells rod-shaped. Utilizing dextrose and sometimes other sugars without producing organic acids in appreciable quantity. A single polar or lateral flagellum or 2-4 peritrichous flagella or non-motile. Have a tendency to be gram-negative.

This definition plainly excludes organisms that show morphological variations like branching forms and coccoid stages, with tendency to be gram-positive. It does not exclude non-motile, gram-negative rods, which do not show morphological changes; but because of the above mentioned similarities between some of the latter and saprophytic members of the *Mycobacteriaceae*, it is evident that one should not definitely assign any particular non-motile organism to this family until its relationships have been carefully studied.

ORGANISMS INCLUDED IN THE PRESENT STUDY

The present study included the following groups of organisms:

Rhizobium spp.: six species, producing nodules respectively on cowpea, soybean, alfalfa, clover, pea and bean.

Phytomonas spp.: eight species of plant pathogens, more fully described on p. 216, belonging to that group of plant pathogens producing lesions on roots or stems of plants and not showing acid production from sugars.

Alcaligenes spp.: Several cultures named *Alcaligenes faecalis* and thus supposed to represent the type species of this genus were obtained from various laboratories. Most of them were recent isolations and none had histories to connect them with any previously published description.

Two strains of *Alcaligenes viscosus* (a species which has been placed in this genus in all recent editions of Bergey's Manual) were obtained from the American Type Culture Collection. These were originally furnished by Dr. Hammer of Iowa State College.

The 5th edition of Bergey's Manual has placed the species *Alcaligenes radiobacter* in this genus for the first time. As this species is very similar to the legume nodule organisms (*Rhizobium spp.*) it seemed especially important to include it in this study. Several strains were therefore included, some isolated in the writers' laboratory and others obtained from various laboratories in the United States.

Chromobacterium spp.: Eleven strains of violet chromogenic bacteria, obtained from Dr. Cruess-Callagan of Dublin, Ireland.

Miscellaneous soil forms: 10 or 12 strains of unnamed organisms were isolated from soil. For this purpose a series of isolations was made of non-spore-forming motile rods which produced punctiform colonies in gelatin.

METHODS

Routine tests for ordinary physiological reactions were made on agar slants, because many of the organisms studied grew very poorly in liquid media. Both beef-extract-peptone agar and the synthetic agar listed in the Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists were used.

Daily routine examinations of morphology were made from agar slant cultures for a period of about one week. The ammonium oxalate Gram method described in the above mentioned Manual was used.

Flagella stains were made by the modification of Gray's technic recently proposed by Hofer and Wilson (1938) and recommended by two of the present writers (Conn and Wolfe 1938b) as a routine procedure. All cultures were studied regardless of whether they appeared motile in hanging drop culture or in semi-solid agar. An occasional culture was found that showed the presence of flagella without any other indication of motility.

A few cultures were also studied by smearing the culture over the surface of agar dried on microscopic slides and then at hourly intervals staining a slide with erythrosin. This method (Toppey 1938) is intended to show the presence of branching forms and striking arrangements of the cells in young cultures.

STUDY OF RHIZOBIUM SPP.

If one accepts the grouping of these organisms in the family recognized in the 5th edition of Bergey's Manual as *Rhizobiaceae*, the genus *Rhizobium* becomes the type of the family and deserves primary consideration. Organisms of this genus have long been known and have been intensively studied. They are short, non-spore-forming rods, which are ordinarily gram-negative and show a tendency to branch when growing in the roots of their hosts. This branching tendency has not proved evident, however, when they are studied by the above-mentioned "little plate" technic.

Their flagellation has been discussed for many years; some claimed them to be peritrichous, others monotrichous. It gradually became evident that those who favored the latter view had been studying either the soybean or the cowpea organism, while those who called their group peritrichous had been considering the more rapid growing types, such as those of pea, bean, or alfalfa nodules. Some (e.g. Shunk, 1921; Löhnis & Hansen, 1921) have even suggested putting the latter in the genus *Bacillus* the former in *Pseudomonas*. The sketches in figure 1 make this difference clear. (The cell of the cowpea organism which shows two flagella was an isolated instance and may represent a chance occurrence of a loose flagellum in such a location as to appear attached.) It is very evident that the cells of the monotrichous group are as likely to have their flagellum attached at the side as at the pole; and that similar monotrichous cells occur in the case of pea, bean, and alfalfa cultures. As soon, therefore, as the concept of degenerate peritrichous flagellation is accepted, it is clear that all of these cultures are of the same type. It is interesting, although not necessarily significant, that the more vigorous types possess the more flagella.

In regard to flagellation, therefore, the members of this genus agree with all the rest of the organisms here considered. A further point of agreement is that they are capable under strongly aerobic conditions (see Georgi and Wilson, 1933) of breaking down glucose so completely that 60-80 per cent of its carbon is converted into CO_2 . This is apt to result in no appreciable increase in H-ion concentration unless the organism is growing on synthetic

medium of low buffer content. It should nevertheless, be observed that some authors (Virtanen, *et al.*, 1934; Rajagopalan, 1938) have found measurable quantities of several fatty acids produced from sugar by certain of these organisms, with comparatively low percentage of CO₂-production. Such work, how-

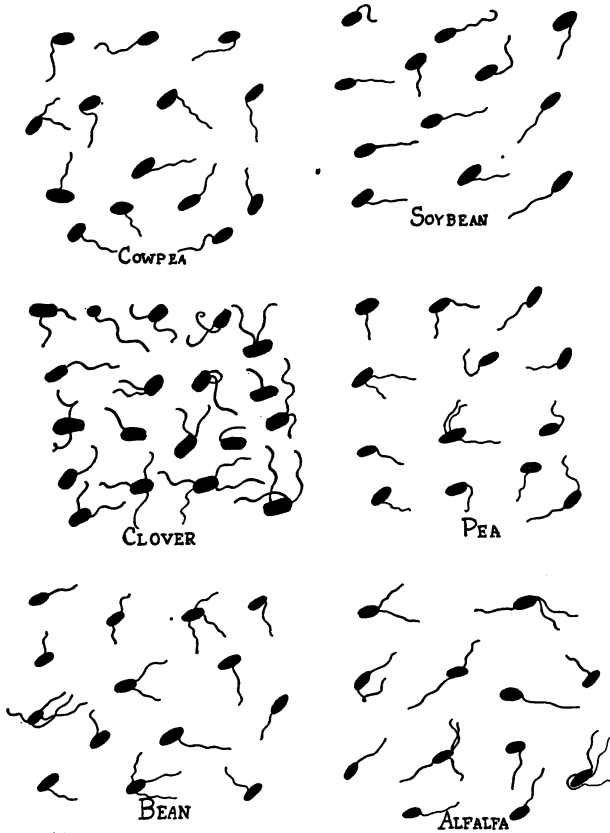


FIG. 1. FLAGELLATION OF RHIZOBIUM (LEGUME NODULE BACTERIA)

ever, has been done with mass inoculation of large quantities of liquid media with large quantities of inoculum, and may well represent incomplete oxidation occurring in the absence of optimum quantities of oxygen.

In addition to these characteristics there are others, which taken together, serve to distinguish the genus *Rhizobium* from

the other genera under consideration, namely: failure to produce nitrite from nitrate in either liquid or agar media; gum formation in the presence of available sugar or alcohols; nodule production on legumes to which the cultures studied are adapted, accompanied by nitrogen fixation in the plant.

STUDY OF CERTAIN PLANT PATHOGENS

In the 5th edition of Bergey's Manual the genus *Phytomonas* is not placed in the family *Rhizobiaceae*; but is divided into three parts and the statement is made that one of them (Appendix II) includes species probably related to the *Rhizobiaceae*, although to avoid renaming of species these have not been moved for the present. This section of the genus includes the crown gall organism and others producing hypertrophies or other root lesions. It has, in fact, been recognized that *Phytomonas tumefaciens* is so nearly like *Rhizobium* that it is very difficult to tell them apart by cultural tests.

For this reason, it was decided to collect a series of cultures of plant pathogens of this group and to see how they compared culturally and morphologically with the other organisms being studied.

The following collection of cultures were obtained:

P. tumefaciens (Smith & Townsend) Bergey *et al.*, 4 strains, including 2 single-cell isolations obtained from the Dept. of Agric. Bacteriology, Univ. of Wisconsin.

P. rhizogenes Riker *et al.* (the cause of hairy root), 1 strain from Dept. of Agric. Bacteriology, Univ. of Wisconsin.

P. fasciens Tilford (hosts, sweet pea and chrysanthemum), 2 strains from Dept. of Plant Pathology, N. Y. State Col. of Agric.

P. insidiosa (McCulloch) Bergey *et al.* (host, alfalfa), 2 strains from Dept. of Plant Pathology, N. Y. State Col. of Agric.

P. michiganensis (Erw. Smith) Bergey *et al.* (the cause of tomato canker), 3 strains from Bureau of Plant Industry, Washington, D. C. This organism has been placed by Jensen (1934) in *Corynebacterium*.

P. gypsophilae, (Brown) Magrow (host, *Gypsophila*), 1 strain from Bureau of Plant Industry, Washington, D. C.

P. pseudotsugae (Hansen & Smith) Burkholder (host, Douglas fir), 1 strain from Dept. of Plant Pathology, Univ. of California.

P. savastanoi, (Erw. Smith) Bergey *et al.* (the cause of olive knot), 2 strains from the Citrus Experiment Station, Riverside Calif., and several fresh isolations from olive knots, whose pathogenicity has not been tested.

These cultures were found, as anticipated, to be in some cases quite similar to *Rhizobium spp.* *Phytomonas tumefaciens* is the most like the legume nodule bacteria, agreeing even in the matter of producing gum on sugar media; this species, together with *P. rhizogenes*, *P. savastanoi*, and *P. gypsophilae* show the same type of flagellation that characterizes the genus *Rhizobium*, as well as other characteristics described above under that genus. The fact that they are usually described as having polar flagella is undoubtedly due to the ease with which "degenerate peritrichous flagellation" can be misinterpreted. Of three strains of *P. michiganensis*, one appears to be non-motile, while the other two have the same type of flagellation as the above. *P. pseudotsugae*, which, like the latter is generally described as non-motile, seems to have two polar flagella; while *P. fasciens* and *P. insidiosa* have as yet shown no sign of motility or of possessing flagella. The very sluggish motility of the motile organisms in this group and the difficulty of staining their flagella make it at least doubtful as to whether it may not be possible in the future to demonstrate flagella on some of those which are still thought to be non-motile. All these organisms agreed in producing no detectable acid on a peptone sugar medium, and all but two (*P. insidiosa* and *P. gypsophilae*) produced abundant CO₂ from glucose. Although all of them are generally assumed to be gram-negative, several showed a distinct tendency at times to be positive.

The flagellation of *Phytomonas tumefaciens* and *P. rhizogenes* is shown in figure 2. Especially interesting is the fact that both of the strains of the former derived from single-cell isolation were definitely monotrichous, as illustrated by the two left-hand sketches in the figure. Many such monotrichous cells were present in preparations from other cultures; but the same slides would also show cells with as many as four flagella. This suggests

that in the case of this degenerate peritrichous flagellation, it may be possible to separate out by single-cell culture strains having a constant number of flagella. More study of this interesting possibility seems desirable.

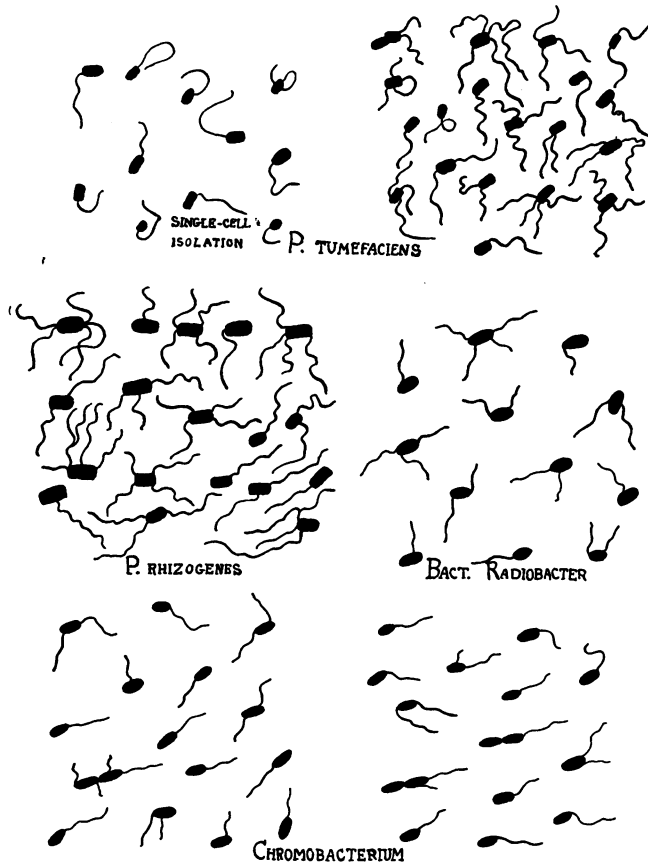


FIG. 2. FLAGELLATION OF TWO PLANT PATHOGENS, BACTERIUM RADIOBACTER, AND CHROMOBACTERIUM

It seems probable that of these plant pathogens, those species which are most similar to the legume nodule bacteria should be placed in close relationship to the other organisms of the *Rhizobiaceae*. Whether this should be done with the non-motile organisms is questionable. *P. pseudotsugae*, with its polar flagella, does not seem at present to belong with the organisms here

under consideration; although it is realized that determinations of flagellation of these very small organisms with only 1 or 2 flagella are difficult to make and that future study may show it less different from the others in this group than it seems at present.

POSITION OF *BACTERIUM RADIOBACTER*

It has long been realized that there is a soil organism frequently associated with roots which is similar to *Rhizobium* spp. and often confused with the legume nodule bacteria, *Bacillus radiobacter* Beijerinck and Van Delden. It is ordinarily called *Bacterium radiobacter* but in the 4th edition of Bergey's Manual was placed in *Achromobacter* and in the 5th edition transferred to the genus *Alcaligenes*. It was hoped that the present study might show the most logical position for this organism.

Recent studies in this laboratory have confirmed the impression that the cultural characteristics of this organism are practically identical with those of *Rhizobium* spp. and *Phytomonas tumefaciens*. Morphologically, it is almost identical with the species of *Rhizobium* having larger cells, and has the same type of flagellation; see figure 2. This has brought about as much confusion in the literature concerning the arrangement of its flagellation as has occurred in the case of *Rhizobium* spp.

The similarity between this organism and *Rhizobium* spp. is most striking in the case of the alfalfa organism. The only consistent difference between these two organisms is that *Bacterium radiobacter* is unable to produce nodules on any legume, but is able to grow in media with a reaction of pH 10 to 11, while none of the legume nodule organisms grows in such alkaline media.

The decision to put it in the genus *Alcaligenes* is not at all illogical as that is the only genus recognized in Bergey's Manual except *Achromobacter* which has the combination of characteristics shown by this organism; and *Achromobacter* is an ill-defined genus with a possibly unrecognisable type species.

THE GENUS *ALCALIGENES*

This decision makes it important to determine whether the type species of *Alcaligenes* can be recognized. No one seems to ques-

tion but that the type of this genus is *Alcaligenes faecalis* (*Bacillus faecalis alcaligenes* Petruschky). The original description of this organism makes it clear that it is a peritrichous, non-spore-forming rod of intestinal origin, producing no acid in sugar broths and causing alkalinity in milk. It has occasionally been described as monotrichous. None of these characteristics except its intestinal origin serves to distinguish it from any of the organisms just discussed. Accordingly, it seemed well to secure cultures from different sources and to see whether any agreement could be found among them. Twenty-two cultures were compared, all of which are comparatively recent isolations and make no claim to date back to the original description. The oldest of these were obtained from the American Type Culture Collection and were isolated less than 20 years ago.

One or two of the cultures were fluorescent with a tuft of polar flagella and were obviously incorrectly named. All the rest agree with Petruschky's original description of the organism; but the cultures seemed to include two distinctly different types. There is apparently considerable disagreement as to the identity of this species, so far as can be judged by the cultures collected. It is especially to be noted that two of the three cultures obtained from the American Type Culture Collection differed from all but one of those received from other sources in that they proved capable of making vigorous growth and producing abundant CO₂ in a synthetic glucose medium. Two of the 22 cultures received were apparently non-motile and two others had polar flagella; but by far the majority showed what the authors are denoting "degenerate peritrichous flagellation." All but a small minority of the cultures, however, agreed in the following characteristics: they produced alkalinity without peptonization in litmus milk; they grew poorly in synthetic glucose media but growth was greatly stimulated by the addition of a small amount of yeast extract; there was no evidence of any acid from sugar except CO₂ but some of the cultures produced this in abundance in a glucose medium suitable to their growth. About half the cultures studied produced nitrite from nitrate, apparently differing in this respect from the description of this species in the

5th edition of Bergey's Manual; but because of disagreements noticed according to whether the test was made in a synthetic medium or a medium containing peptone, it is suspected that this characteristic varies within the species. Presumably the

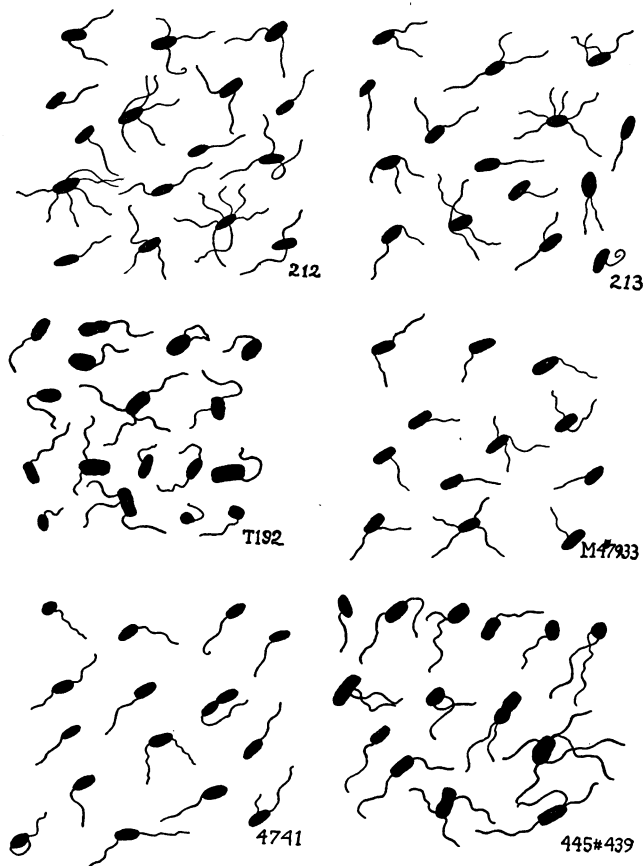


FIG. 3. FLAGELLATION OF SIX STRAINS OF *ALCALIGENES FECALIS*
 Strains 212 and 213 are of the type growing well on synthetic media; other four strains unable to do so.

organism is a nitrate producer showing nitrite under some circumstances but not under others. In all other respects the organism should be described as in Bergey's Manual.

Apparently, therefore, there is a commonly recognized organ-

ism to which the name *Alcaligenes faecalis* can be assigned. One should, however, be cautious not to call by this name all intestinal organisms producing alkalinity in litmus milk and no acid from glucose, as there seem to be other types. Apparently,



FIG. 4. FLAGELLATION OF MISCELLANEOUS UNNAMED GRAM-NEGATIVE, NON-SPORE-FORMING ORGANISMS FROM SOIL

moreover, the same species may occur in other habitats; one strain from fermenting sauerkraut and one from a Cuban sugar mill proved impossible to distinguish from the typical *Alcaligenes faecalis* cultures.

It should be added that many soil bacteria, whose identity has not been worked out as yet, show the same type of flagellation as

A. faecalis. Figure 4 presents six such strains. These organisms are also similar in physiology, although they grow better in synthetic media; it is difficult to point out any feature in which they differ markedly from this type species.

A STUDY OF CHROMOBACTERIUM

Because statements in the literature have sometimes recorded the violet chromogens as peritrichous and sometimes as monotrichous, they were included in this study. These organisms have in recent classifications of bacteria ordinarily been put in the genus *Chromobacterium*. Cruess-Callaghan and Gorman (1935). These authors recognized three species, one of which (*Bacterium janthinum*) was peritrichous, a second (*Bacterium violaceum*) as having one polar flagellum, while *Bacterium violaceum-amethystinum* showed both types of flagellation. Five strains labelled *Bacterium violaceum* and seven denoted as *Bacterium violaceum-amethystinum* were secured for the present work. These strains were originally obtained from the authors of the paper cited.

All of these twelve strains were found to be gram-negative rods having physiological characteristics very similar to the organisms above discussed. Acid production was seldom observed in a sugar medium containing peptone, but in a synthetic medium acid ordinarily appeared with glucose and sometimes with sucrose. CO₂ was observed in Eldredge tubes in media containing glucose, but the amount was never large. All but one of the twelve strains showed rather weak nitrite production from nitrate.

All five strains labelled *Bacterium violaceum* had a single flagellum which seemed to be polar. This same observation was ordinarily made for those cultures labelled *Bacterium violaceum-amethystinum*, except that three of the strains sometimes showed cells with two flagella. Two of these are shown at the bottom of figure 2. When two flagella were observed they were never both at a pole. These observations agree with those of Cruess-Callaghan and Gorman.

The grouping of these organisms, therefore, still remains a little uncertain. They are certainly like the other organisms

discussed above in physiology, except for the fact that they do not produce large amounts of CO₂ in any medium yet studied. In the matter of flagellation they differ slightly from most of the others in that, whenever a single flagellum has been observed it has usually been attached at the pole, seldom laterally. This makes it a little doubtful whether these organisms display degenerate peritrichous arrangement of the flagella. Nevertheless, the occasional occurrence of cells with two flagella, which do not occur in a polar tuft, suggests that placing the organisms in the *Rhizobiaceae* (as done in the 5th edition of Bergey's Manual) is as satisfactory an arrangement as can be worked out at present.

CONCLUSIONS

Lochhead and Taylor (1938) have recently stated that the soil organisms of the general type here considered are very unstable physiologically and show a large amount of adaptability to changing conditions. For this reason, they regard them as difficult to classify on a physiological basis. With this point of view the present writers are in entire agreement. It is considered that progress will be made in the classification of these organisms largely by taking into account such points as Gram's reaction, type of flagellation if any, and tendency to show true or false branching or irregularities in shape of cells.

If one leaves *Bacterium globiforme* out of consideration, and regards the gram-positive simple rods as belonging with the branching forms that are often called *Mycobacterium*, one has left a fairly concrete group which can be considered as the family *Rhizobiaceae* and defined as follows:

Short rods, usually under 0.8 micron in transverse diameter, prevailingly gram-negative; no endospores; flagella sometimes lacking, sometimes 1-4 in number, but, when more than one, never occurring in a polar clump; capable of utilizing glucose without the production of organic acid, with CO₂ and cell substance the principal carbonaceous end-products (the amount of acid produced rarely being enough to become evident in a well-buffered medium). Type genus, *Rhizobium*, which includes the legume nodule organisms.

It is recommended that this family should be made up as follows:

Genus 1. Rhizobium, containing the legume nodule organisms. Type species: *R. leguminosarum* Frank. Possibly future study will show the species usually called *Bacterium radiobacter* (Beijerinck & Van Delden) Löhnis (recently placed in the genus *Alcaligenes*) to be a free-living, non-N-fixing species of this genus.

Genus 2. Chromobacterium, containing primarily the violet chromogens. Type species *C. violaceum* (Schroeter) Bergonzini.

Genus 3. Alcaligenes, consisting of non-chromogenic saprophytes. Type species *A. faecalis* (Petruschky) Castellani and Chalmers. The limits of this genus need careful study, as there are numerous soil and milk forms that differ so slightly from the intestinal inhabitant which is the type species that it is still difficult to tell just where to draw the line between this and similar genera.

Appendix. Certain plant parasites, notably the species now designated as *Phytomonas rhizogenes* and *P. tumefaciens*, are very closely related to species in the genera *Rhizobium* and *Alcaligenes*. There seems little question but that they belong in this family. Whether they should be put in a new genus by themselves or in one of the above is a matter for future study.

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