SOIL BACTERIA SIMILAR IN MORPHOLOGY TO MYCOBACTERIUM AND CORYNEBACTERIUM[1]

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When Lehmann and Neumann (1896) first proposed the genera *Corynebacterium* and *Mycobacterium*, the former was intended primarily for the diphtheria organism and the latter for the tubercle and leprosy organisms. In recent years there has been a tendency to broaden both of these genera to include, on the one hand, almost any species showing morphological irregularity and, on the other, various gram-positive nonsporeformers even though showing little or no irregularity in morphology. The original descriptions of these genera were very simple and included only the following essential characters:

Mycobacterium. Slender rods with some branching; acid-fast; colonies on agar, dry, wrinkled. Type, *M. tuberculosis.*

Corynebacterium. Rods with ends often swollen and club-shaped, banded with alternate streaks of stain, sometimes developing filaments and true branching (by implication non-acid-fast, although this characteristic is not definitely mentioned by the authors until a later edition of their book); growth on agar, soft and nonadherent. Type, *C. diphtheriae.*

Various other characteristics have been listed by later authors for the genus *Corynebacterium*, the most important of which is the so-called "snapping division" of the cells. As this feature is difficult to observe directly, it is usually inferred from the orientation of the cells as described by Kisskalt and Berend (1918), i.e., a tendency to pile up in heaps, with palisade or V-form arrangement. Stress on this characteristic by later authors has undoubtedly been responsible for some unwarranted broadening of the genus, as orientation of this sort can often be observed and does not necessarily indicate the type of cell division which is supposed to be characteristic of *Corynebacterium.*

As a matter of fact, broadening of the two genera has taken place in several directions until they have come to overlap. Moreover, each genus has had species assigned to it which seem to differ more from other species in the same genus than does the type species of one genus from the type of the other. This broadening has taken place along the following lines:

Mycobacterium. (1) The inclusion of all acid-fast forms, whether or not branching occurs. (2) The inclusion of many branching forms (Krassilnikov, 1934) whether or not they are acid-fast.

Corynebacterium. (1) The inclusion of a rapidly expanding group of "diphtheroids," i.e., animal parasites which are gram-positive and show the type of orientation described by Kisskalt and Berend; a few of these are anaerobic. (2) The inclusion of certain gram-positive plant pathogens, following the lead of

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Jensen (1934). (3) The inclusion (also following Jensen, 1934) of strongly aerobic soil bacteria or extremely irregular morphology, showing coccoid and branched forms as well as rods.

The authors' interest in these genera comes from the fact that one of them (Conn, 1928) described under the name of *Bacterium globiforme* an organism which appears as gram-negative, short rods in 24-hour agar slant cultures but as gram-positive cocci after the cultures are 3 to 4 days old. Cultures of this organism have been sent to Krassilnikov and to Jensen; the former is sure it is a species of *Mycobacterium*, the latter that it is a *Corynebacterium*. The latter opinion has been indorsed by Lochhead, who, with one of his associates (Taylor and Lochhead, 1937; Taylor, 1938; Lochhead, 1940), has become one of the leading students of organisms of this type. Their impression in the matter has been summed up by Lochhead (1940) in the following words: "The characteristic *Bact. globiforme* is now believed by us to represent a special group of the corynebacteria with distinctive cultural and physiological properties."

When *Bacterium globiforme* was first described, its author was not unaware of certain resemblances between this organism and either *Mycobacterium* or *Corynebacterium*; but it appeared different in so many ways from the type of either genus that the resemblances were regarded as probably superficial. It was then named as a species of *Bacterium* because that genus was then regarded by the author as a grouping place for species whose relationships were not definitely understood. Since then, however, the conception of *Bacterium* has changed, and it is now usually defined so as to exclude an organism with morphology like that of the species in question.

It must be remembered that when this species was first described the idea of life cycles involving changes in morphology had not been fully accepted, and it took some courage to describe an organism appearing as a gram-negative rod in one stage and a gram-positive coccus in another. Furthermore, the old ideas of monomorphism were then so persistent that it did not occur to the author to make a sufficiently intensive study of the organism to learn whether other morphological forms occurred in its life cycle.

Work on organisms of this type was dropped in the writers' laboratory for several years. It has recently been resumed with the object of comparing cultures of the *Bacterium globiforme* type with strains from other laboratories that have been named as species of *Corynebacterium* or *Mycobacterium*, with the hope of learning how close the relationship between them may be.

EXPERIMENTAL WORK

Mycobacterium Cultures

No extensive study was made of soil cultures that could be regarded as species of *Mycobacterium*. Four cultures, however, were obtained from Jensen labeled, respectively, *Mycobacterium coeliacum*, *M. convolutum*, *M. rubropertinctum*, and *M. crystallophagum*. No similar organisms were found among the available collection of cultures isolated from local soils on ordinary media without special enrichment technique. An attempt was made to secure such forms by isolating

in media to which paraffin-coated pebbles were added, a technique which is regarded as favoring the development of acid-fasts; a few partially acid-fast organisms were found, but so late in the work that no careful study of them has yet been made.

The four cultures obtained from Jensen all showed a slight tendency to branch, although not so much variation in morphology was observed as in the organisms to be discussed in the following pages. Three of them were acid-fast, although *M. crystallophagum* was not. All four were gram-positive. They all grew on Mueller's tellurite agar (Difco dehydrated), with typical blackening. All four grew on agar with ammonium phosphate as a sole source of nitrogen; and none of them showed diastatic action on starch.

The authors do not yet feel their work on this group has been extensive enough to warrant an opinion where in the scheme of bacterial classification these organisms belong. It should be remarked that certain students of the pathogenic acid-fasts (e.g., Gordon and Hagan, 1936) regard soil acid-fasts as very closely related to the pathogens. Accordingly it seems quite likely that Jensen has been entirely justified in describing such forms as species of *Mycobacterium*. It should be emphasized again, however, that Krassilnikov's "mycobacteria" (whose reaction to the acid-fast stain has never been described) do not seem to belong in the genus, but appear rather to be related to the types described below.

Corynebacterium Cultures

In order to learn how closely the soil bacteria of the *Bacterium globiforme* group are related to *Corynebacterium*, it seemed desirable to obtain a collection of cultures that have been assigned to that genus. The following cultures, as representing what other workers think should go in the same genus as the diphtheria organism, were obtained: 11 cultures of animal and human parasites of diphtheroid nature obtained from P. R. Edwards of the University of Kentucky, W. A. Hagan of Cornell University at Ithaca, H. E. Morton of the University of Pennsylvania, M. Frobisher of the Johns Hopkins Medical School; three strains of *C. helvolum*[2] and one of *C. tumescens* (both soil organisms) from Jensen; and four plant pathogens that have been put in the genus—*C. flaccumfaciens* and *C. fascians* from W. H. Burkholder of Cornell University at Ithaca, *C. poinsettiae* from M. P. Starr of Brooklyn College, and *C. michiganense* obtained many years ago from Miss Bryan, then in the Department of Agriculture at Washington.

Animal diphtheroids. The animal and human diphtheroids showed greatest similarity to the type of the genus, *Corynebacterium diphtheriae*. These organisms are comparatively constant in morphology, appearing generally as

[2] Jensen regards this species as synonymous with Zimmermann's *Bacillus helvolus*, renamed *Corynebacterium helvolum* by Kisskalt and Berend. As there is no evidence that Jensen received any strain of Zimmermann's organism for comparison, it is preferred here to think of Jensen's *C. helvolum* as an emendation of the earlier species which stands only if Zimmermann's original organism can no longer be identified. See description at the end of this article.

rods, which are sometimes slightly wedge-shaped or club-shaped, although this morphological peculiarity is not ordinarily as pronounced as in the diphtheria organism itself. The palisade or zigzag arrangement of the cells is common, but truly branched cells have not been observed in the present investigation. The organisms are ordinarily gram-positive; or if gram-variable, the tendency is for the young cells to be positive, the older ones negative. In physiology, the most striking feature is inability to grow on any synthetic medium investigated, a fact which indicates their need of some organic form of nitrogen, or of accessory growth factors, or both. They do not liquefy gelatin or have any visible action on milk, but they are strong producers of acid from sugar.

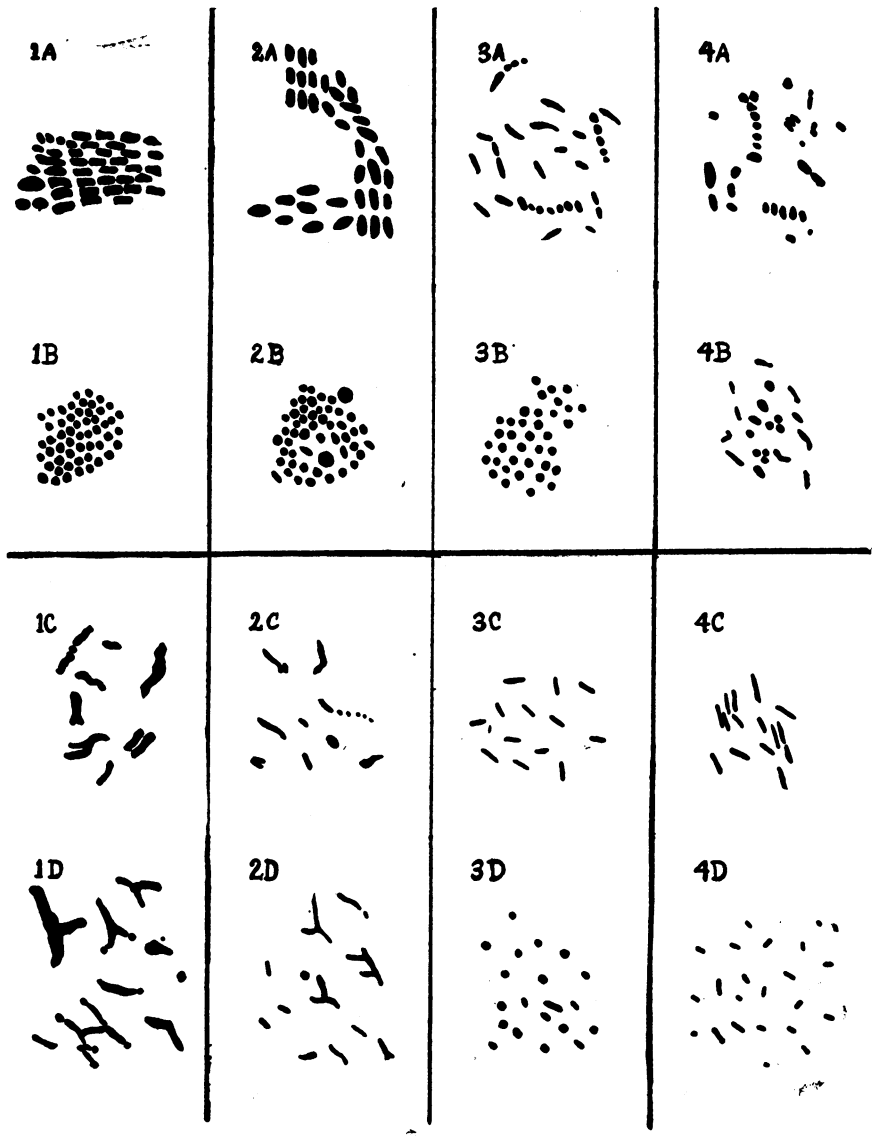
Soil organisms. The soil organisms (Jensen's cultures) proved distinctly different. Their morphological variations are greater, as they often show true branching of the cells, frequently with conidialike spherical bodies on the ends of the branches, and often with the production of larger coccoid bodies (called cystites by Jensen). (See figures 2 and 3.) They are gram-variable, and unlike the animal diphtheroids (figure 4) their tendency is for the older cells to be more strongly positive than the younger. The significance of this behavior to the gram reaction should be more carefully studied in relation to the present-day conceptions of the reaction as dependent on ribonucleic acid; but offhand one would say that there is a significant difference between species which tend to become gram-positive only in older cultures and those which tend to become *less* strongly so in older culture.

Another striking difference between Jensen's "corynebacteria" and the animal diphtheroids is their ability to grow on synthetic media, with ammonium salts, nitrate, or urea as a sole source of nitrogen, and without the addition of growth accessory factors. They liquefy gelatin and digest milk, but show only weak production of acid from any sugar.

These differences appear to the writers as being of sufficient significance to justify removal of Jensen's species from the genus *Corynebacterium*. Their striking morphology, however, is enough like that of *Mycobacterium* and possibly even of *Nocardia* (Jensen's *Proactinomyces*), that they should be kept close to these genera rather than included in the *Eubacteriales*.[3]

Plant pathogens. The plant pathogens present a rather more complicated situation. It has long been recognized that the gram-positive plant pathogens do not fit properly in the genus *Phytomonas*, where several of them have been placed in the past, nor for that matter in any other available genus. Jensen (1934) apparently was the first to place one of them definitely in *Corynebacterium* (i.e., *C. michiganense*). Dowson (1942) transferred two other species (previously *Phytomonas fascians* and *P. flaccumfaciens*) to that genus. This step having already been taken by earlier writers, it was natural for Starr and Pirone (1942)

[3] It should be observed that some recent classifications (e.g., in the forthcoming sixth edition of *Bergey's Manual*) place *Corynebacterium* in the *Eubacteriales*, *Mycobacterium* in the *Actinomycetales*. This arrangement, however, does not agree with the writers' opinion as to the actual relationships of the organisms. See the section of this article on taxonomic considerations.



FIGS. 1–4. SKETCHES TO SHOW PREVAILING MORPHOLOGICAL TYPES ON AGAR AND IN LIQUID MEDIA, IN 1- AND 4-DAY CULTURES

Fig. 1. *Arthrobacter globiforme.* Fig. 3. *Arthrobacter tumescens.*
Fig. 2. *Arthrobacter helvolum.* Fig. 4. *Corynebacterium equi.*

The individual sketches are arranged in rows as follows: Row A, 24-hour agar slant culture; Row B, 4-day agar slant culture; Row C, 24-hour culture in sauerkraut glycerophosphate medium; Row D, 4-day culture in sauerkraut-glycerophosphate medium. Preparations shown in rows A and B stained with crystal violet; those in rows C and D with Benian's Congo red method.

on describing a new gram-positive species of a plant parasite (*Phytomonas poinsettiae*) to suggest that it might also be placed in *Corynebacterium*. The

present writers have obtained cultures of all four of these organisms to see whether they are related to any of the other species that have been placed in the genus, and if so to which ones.

Briefly, the writers' conclusions are that all four species (with the possible exception of *Corynebacterium michiganense*) show such differences, both morphologically and physiologically, from the type of that genus, that they clearly belong elsewhere. In fact, they show such differences among themselves that perhaps they do not all belong in the same bacterial genus. Physiologically these plant pathogens seem to stand between the animal diphtheroids and the soil forms mentioned above: they grow on synthetic media, but their proteolytic action is weak or absent. In morphology they differ so much from one another that further discussion is necessary.

Corynebacterium michiganense is definitely a nonmotile, gram-positive rod, most nearly like typical diphtheroids of any of the four, although it shows but slight tendency to develop club-shaped forms or other irregularities of morphology. Like the animal diphtheroids, it does not liquefy gelatin, and in fact shows enough similarity to the latter so that Jensen's transfer of this species to *Corynebacterium* may perhaps be justified.

Corynebacterium fascians is a nonmotile, gram-variable rod, with a tendency to be more strongly gram-positive in young culture than in old. Although it shows very little morphological variation, it has an appearance on agar slant (dry and yellowish) which strongly suggests relationship to some of the *Actinomycetaceae* (certain *Nocardia* species, for example). As it is slightly proteolytic, it is less like typical *Corynebacteria* forms than the preceding species. Its inclusion in the genus is at least questionable.

Corynebacterium poinsettiae and *C. flaccumfaciens* are also yellow chromogens; they are both gram-positive, but are motile with single polar flagella. The latter species often shows sickle-shaped cells with a single, unusually long flagellum at the pole, and one would place the species unquestionably in *Vibrio* if it were not gram-positive. The inclusion of these forms in *Corynebacterium* is highly dubious. The genus is typically one of nonmotile species, and there is little, if any, justification for including types with polar flagella. Such a statement, however, need not reflect on those who proposed placing them in *Corynebacterium*; Jensen, as just shown, has good justification for transferring *Phytomonas michiganensis* to *Corynebacterium*; and Dowson as well as Starr and Pirone could point out close resemblances between that species and the other gram-positive plant pathogens. Nevertheless, *C. poinsettiae* and *C. flaccumfaciens* are distinctly different from the typical *Corynebacterium* species, on the one hand, and from soil forms (as typified by Jensen's cultures), on the other. The present writers hesitate to say just how they should be placed; further study of the question seems indicated.

Cultures from Local Soil

To compare with the above-mentioned cultures obtained from other laboratories, some 32 strains of organisms like *Bacterium globiforme* in morphology,

isolated from local soils, were studied. Included among them were 14 strains that had been carried in stock for years; the rest were fresh isolations. In comparing these with the cultures from other laboratories it was desired to see whether they belonged in *Mycobacterium* (after Krassilnikov) or in *Corynebacterium* (after Lochhead and Jensen); or if in neither, where they should be placed taxonomically.

A brief study was enough to convince the writers that acceptance of Krassilnikov's conception is out of the question. These cultures can scarcely be called *Mycobacterium*, chiefly because they show no evidence of acid-fastness. Also, they show rather more tendency to branch than typical members of that genus; in this they somewhat resemble *Nocardia* in morphology, but differ from it in having smooth, soft growth on agar (like ordinary bacteria) rather than the dry, wrinkled growth suggestive of the tubercle organism. The fact that Krassilnikov called a culture of *Bacterium globiforme* a species of *Mycobacterium*, whereas Jensen states that the same culture belongs in *Corynebacterium*, is strong evidence that the former's *Mycobacterium* is equivalent to the latter's *Corynebacterium*. Jensen's conception seems more acceptable than Krassilnikov's. As a matter of fact, the similarity of the cultures isolated from local soils to Jensen's *Corynebacterium helvolum* is so great that careful study was needed to show that there really are distinct differences.

Morphology. Practically all the cultures selected for this comparative study showed the morphological growth cycle on agar which has been described in the past as characteristic of *Bacterium globiforme*, and is illustrated by the photomicrographs of Conn (1928, p. 6) as well as by sketches 1A and 1B in this paper. Briefly, it may be said that the organisms appear as gram-negative rods in 1-day culture, and as prevailingly gram-positive cocci in older cultures. This particular type of morphology is chiefly characteristic of agar slant cultures. In liquid media the rods tend to elongate and branch, as shown in figures 1C, 1D, and 5. These branching forms are most easily shown by the Benians' negative stain, using Congo red turned blue by treatment with acid; with this technique the apparent diameter of the cells is smaller than when they are positively stained in dry condition, a difference that shows in the sketches of figure 1. Sometimes in liquid cultures about 24 hours old, the nodes of these branched forms appear swollen; and when a gram stain is made of such cells, the swelling proves to be due to a gram-positive coccoid body at the node, the rest of the cell being gram-negative (see figure 5). According to Krassilnikov (personal correspondence) these structures are actually germinating spores. In older liquid cultures similar coccoid bodies seem to be borne like conidia on the ends of the branches, and it can be shown that these are also gram-positive. It is still uncertain whether both of these types of spherical bodies are identical, or whether they are the same as the coccoid forms which show in older cultures on solid media. Krassilnikov's interpretation of the matter, on examination of cultures from this laboratory, is that the organism goes through a regular life cycle: coccoid arthrospores; germinating forms with several branches radiating from the remains of the spore; long rods with a tendency to branch; shorter rods; and finally by a process of

further shortening, the breaking up into coccoid arthrospores. No actual demonstration of such a life cycle has been made here, and clearly no such cycle does occur on agar where only rods and cocci are observed. Moreover, if the forms shown in figure 5 are merely germinating spores, it is difficult to explain how the remains of the spore can retain its gram-positive nature while the rods developing from it are gram-negative. Furthermore, although these forms are observed regularly in the cultures regarded here as typical of what has been called *Bacterium globiforme*, other types apparently closely related show no stages except the rods (more or less elongated and more or less irregular in shape) and the cocci. Another guess, which is probably as justified as that of Krassilnikov's, is that the conidialike bodies formed at the ends of the branches are the same as those seen on old agar slants, whereas those formed at the nodes (which are somewhat larger) are another type of spore similar to what Jensen calls cystites.

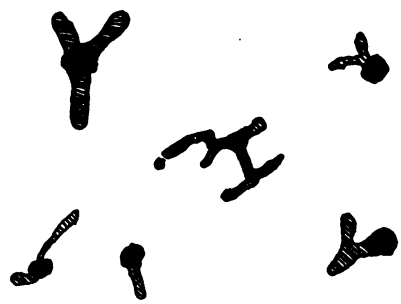


FIG. 5. ARTHROBACTER GLOBIFORME, 24-HOUR CULTURE IN SAUERKRAUT GLYCEROPHOSPHATE MEDIUM, STAINED BY THE GRAM METHOD
Gram-negative structures are shaded; gram-positive structures are solid.

It will be seen from figure 2 that the morphology of Jensen's *Corynebacterium helvolum* is similar. The chief difference is the occurrence of the larger spherical bodies ("cystites") and the persistence of some rod-shaped cells in old agar cultures. Jensen's *C. tumescens* is quite different (figure 3). No other cultures of Jensen's have been available to the authors, but the illustrations in his paper make it evident that *C. helvolum* is the one showing the greatest morphological similarity to what has been recognized here as "*Bacterium globiforme*." Cultures exactly agreeing with Jensen's have not been isolated from local soils.

Physiology. When organisms having this type of morphology were first recognized, it was realized that they showed little difference among themselves in physiology; but as nearly all the physiological characteristics were negative ones (except gelatin liquefaction, which was always positive), it was not felt that this apparent similarity was significant. One positive characteristic was the production of small amounts of acid on synthetic media. Jensen lays considerable stress on this; in fact he regards his organisms as distinctly different from *Bacterium globiforme*, because he finds low pH values in carbohydrate media inoculated with *Corynebacterium* strains, whereas *Bacterium globiforme* has been described as producing little acid. It should further be mentioned that it was thought at one time in this laboratory that cultures of this organism could be separated into two species, one producing acid from lactose, the other failing to do so. Subsequent work has shown that none of these differences are of

significance. All of the forms under consideration, regardless of whether they are called *Corynebacterium*, *Bacterium globiforme*, or some other name, can show low pH values after growth on nonbuffered carbohydrate media. It is felt, however, that because of the small amount of actual acid (probably largely CO_2) indicated by the pH changes in the absence of buffer, and because of variations observed in the same cultures on repetition of the tests, such acid production is of no significance and is certainly of no value in the separation of species in the group.

Taylor (1938) divides his cultures (all of which he regards as representatives of *Bacterium globiforme*) into two types: type I utilizes either NO_3 or urea as a sole source of nitrogen, but type II does not grow on a medium containing either of these nitrogen sources, glucose, and mineral salts. The present writers have observed no such distinction. All of the cultures they have found showing the typical morphology described above grow in media having no nitrogen other than one or the other of the two compounds in question. This either means that no representatives of Taylor's type II have been found locally or that the distinction observed by him has failed to appear under the writers' conditions. It should be remarked that among all the cultures studied here, Jensen's *Corynebacterium tumescens* is nearest like Taylor's type II, but it proves, when in vigorous condition, to be able to utilize either NO_3 or urea nitrogen.

Another characteristic of the organisms that was at first thought to be of value for classification is the reduction of nitrate to nitrite. Recent investigation, however, indicates that all the organisms of this group do reduce nitrate and that nitrite production can be detected if a synthetic medium of the right consistency is employed (Dimmick, to be published).

At one time in the course of the investigation it was hoped to make use of bacteriophage typing as a means of separating species from one another in this group. This method had, in fact, proved to have value in classifying certain other soil bacteria (Conn, Bottcher, and Randall, 1945). It did not, however, prove adaptable to the group under investigation, either because of lack of specificity in the bacteriophage, or because of easily developed resistance by the bacteria, or both. It was accordingly given up as a criterion for classification.

Recent study has shown one biochemical test which may be constant enough to separate the cultures into two groups—diastatic action on starch. If this characteristic proves constant on further study, a new species must be made for those forms which do not show such action. Also there are some cultures that are yellow chromogens and that may be a distinct species. Because of the extreme variability in physiology shown by these organisms, however, no such species are made at the present time.

TAXONOMIC CONSIDERATIONS

As explained above, it is felt that Jensen was mistaken in placing such forms in the genus *Corynebacterium*, because there are striking differences between these organisms and the type species of this genus (the diphtheria organism). Morphologically, however, they show greater similarity to *Mycobacterium* and *Corynebacterium* than to eubacteria. Undoubtedly, therefore, they belong in the

Mycobacteriaceae, in spite of certain morphological resemblances to *Nocardia* (*Proactinomyces*). There does not seem to be any genus which exactly fits them in any present system of bacterial classification.

The writers propose for this purpose to revive, by emendation, an old name, *Arthrobacter* Fischer (1895), which as originally proposed was a *nomen nudum*, as no species were named and it was subsequently abandoned even by its author. It is not inappropriate for the present purpose, as it was defined by Fischer as including all nonflagellate, rod-shaped bacteria which produce "arthrospores" as recognized by DeBary. Just what DeBary's arthrospores may have been is not certain, and Fischer later expressed some doubt as to their actual nature; but as the term has been recently revived as a possible name for the conidialike bodies observed in the bacteria now under consideration, an emendation of Fischer's name to apply to them seems permissible.

To discuss the relation of this emended genus to *Mycobacterium* and *Corynebacterium*, certain general points of bacterial classification must be considered. In this the writers prefer to follow the classification given in the fifth edition of *Bergey's Manual*, rather than that which is to be used in the forthcoming sixth edition. This choice is made, first, because the latter classification has, at the time of writing, been distributed only in mimeograph form and, secondly, because in the grouping to be employed in the sixth edition, *Corynebacterium* is placed in the *Eubacteriales* and *Mycobacterium* in the *Actinomycetales*, and the writers prefer to regard these two genera as closely related. According to the fifth edition of *Bergey's Manual*, the differences between these groups may be defined as follows:

A. Simple and undifferentiated forms, without true branching. Occur as spheres, short or long straight rods, or as curved rods..... *Eubacteriales*

B. Cells rod-shaped, clubbed or filamentous, with decided tendency to true branching. Conidia may be formed..... *Actinomycetales*

I. Rods, or filaments with only slight branching. True conidia not formed ..... *Mycobacteriaceae*

II. Filamentous forms, often branched, sometimes forming mycelia. Conidia often present..... *Actinomycetaceae*

The family *Mycobacteriaceae*, as described above, may in the writers' opinion be divided into at least the following three genera:

I. Aerobic slender rods, nonmotile, wholly or partially acid-fast; gram-positive; sometimes clavate or cuneate, or occasionally with rudimentary branching. Many species pathogenic to animals.

Mycobacterium L. and N.

II. Aerobic to microaerophilic rods, ordinarily nonmotile, non-acid-fast; gram-positive (most strongly so in young culture); cells often irregularly shaped, clavate, cuneate, or with rudimentary branching, often beaded or barred. Ordinarily require organic nitrogen, growth accessory factors, or both; typically animal parasites, but some dairy forms, possibly some plant pathogens[4]..... *Corynebacterium* L. and N.

[4] If all but the animal parasites ("diphtheroids") are removed from this species, the authors can see no objection to its transfer to the *Eubacteriales*, as proposed for the sixth edition of *Bergey's Manual*.

III. Strongly aerobic forms, showing rather complicated morphological life cycles, including rods, cocci, clubs, and branched forms; non-acid-fast; gram-variable (young cells usually negative; the older cells, especially those in coccoid form, usually positive); able to live on inorganic nitrogen without added growth accessory substances; typically soil organisms.
Arthrobacter, Fischer, emend.

The last-named genus can be characterized as follows:

Arthrobacter Fischer, *emend.*

Morphology. Varied, with a tendency to go through a more or less definite life cycle, the most characteristic features of which are gram-negative rods in young cultures and gram-positive coccoid forms (arthrospores?) in old cultures, with intermediate stages that may be clubs, branched forms, or short unbranched filaments. Large (1 to 2 μ) spherical bodies are sometimes observed which have been termed "cystites."

Cultural characteristics. Growth on surface of solid media soft and smooth, not dry and wrinkled or hard and leathery, as ordinarily in *Mycobacterium* and the *Actinomycetaceae*. Colonies on poured plates ordinarily small (punctiform). Growth in broth usually slow and never profuse.

Physiology. Can ordinarily use either ammonium salts or nitrates as sole sources of nitrogen. Can utilize glucose and sometimes other sugars as sources of carbon and energy, but ordinarily without producing sufficient quantities of acid to have appreciable effect on the pH of highly buffered media (e.g., containing peptone). Gelatin usually slowly liquefied. Ordinarily cause blackening of Mueller's tellurite agar.

Habitat. Primarily soil.

Type species. *A. globiforme* (Conn) Conn and Dimmick.

It seems possible at present to recognize three species:

Species 1. *Arthrobacter globiforme* (Conn) *comb. nov.* (*Bacterium globiforme*, Conn, 1928; *Achromobacter globiforme*, Bergey *et al.*, *Manual*, 3d ed., 1930.) See figure 1, A to D.

Rods in young standard agar culture of fairly regular morphology, 0.6 to 0.8 by 1.0 to 1.5 μ, becoming (after 2 to 4 days) cocci of about 0.6 to 0.8 μ; branching forms with similar cocci and also large spherical bodies (1 to 2 μ) in liquid media. Growth vigorous, cream colored (never lemon yellow), on standard agar or on synthetic agar with ammonium salts, nitrate, or urea as the sole source of nitrogen. Diastatic action on starch agar. (Further characterization as given in *Bergey's Manual.*) One of the most abundant organisms in local soil.

(It is possible two other species can be recognized, one differing from the foregoing species in producing lemon yellow on agar, the other in failing to show diastatic action on starch. No names are being assigned to them, however, until the constancy of the differences has been proved.)

Species 2. *Arthrobacter helvolum* (Zimmerman), *emend.* Jensen; *comb. nov.* (*Bacillus helvolus*, Zimmerman, 1890; *Corynebacterium helvolum*, Zisskalt and Berend, 1918; *emend.* Jensen, 1934.) See figure 2, A to D.

The three cultures on which this interpretation of the species is based were

secured from Jensen. It is not at all certain that they are the same as Zimmerman's organism. *Corynebacterium helvolum* Kisskalt and Berend, however, was based on a culture received from Zimmerman, and it seems difficult at present to learn just what species it may have been. The present writers, therefore, prefer to regard Jensen's description as an emendation. Based on Jensen's cultures, the species has the following distinctive characteristics:

Morphology: Similar to *A. globiforme* in young agar slant culture; older cultures appear as mixtures of rods, small cocci, and the larger spherical bodies, never appearing as though a pure culture of a micrococcus, as is typically the case with the foregoing species; in liquid media, appearance is similar to that of *A. globiforme.* Growth on standard agar; usually lemon yellow, although sometimes merely cream color. Moderately strong to weak diastatic action on starch. This species has not been found in local soil.

Species 3. *Arthrobacter tumescens* (Jensen, 1934) *comb. nov.* (*Corynebacterium tumescens*, Jensen, 1934). See figure 3, A to D.

Morphology on standard agar slant similar to that of *A. globiforme*, but rods in young cultures are more irregular; in liquid media the branching forms are rare or absent. Nonchromogenic. No growth on tellurite agar. No diastatic action on starch. Growth rather scanty on either standard or synthetic media.

This species seems to be something like the type II of "*Bacterium globiforme*" recognized by Taylor (1938), although it apparently utilizes urea and NO_3 nitrogen. It has not been found in local soil. The description is based on a single culture obtained from Jensen.

Possible Other Species

Jensen places two other species (*Corynebacterium cremoides* and *C. insidiosum*) in the same group with the last two species named, a group which is characterized by great morphological irregularity. He claims the two species to be synonyms of *Bacterium cremoides* Lehmann and Neumann, and of *Aplanobacter insidiosum* McCulloch. The present writers have never received cultures of these forms and do not know whether they should be placed in *Arthrobacter;* according to Jensen's descriptions they seem to be closer to this genus as here defined than they do to true *Corynebacterium.*

CONCLUSIONS

There has been a tendency within the last ten or fifteen years to place certain soil bacteria and plant parasites in the genera *Mycobacterium* and *Corynebacterium;* this practice seems to have started independently with Krassilnikov and Jensen in 1934.

The present study has made it evident that Krassilnikov's *Mycobacterium* is the same as Jensen's *Corynebacterium* and is not acid-fast. Partially acid-fast organisms, apparently related to *Mycobacterium*, do occur in soil; but as they do not seem to make up part of the predominant soil flora, they have not been included in the present study.

Special attention has been given to forms found in local soils that are similar to

Jensen's group I of *Corynebacterium* (which show much morphological variation and which he claims are most closely related to the diphtheria organism). It is clear that among these forms should be included *Bacterium globiforme* Conn. It is also evident that they differ so much from *Corynebacterium diphtheriae* that, although probably related to it, they scarcely belong in the same genus. For this group of species the name *Arthrobacter*, emended from A. Fischer, is here proposed, with *Arthrobacter globiforme* (Conn) *comb. nov.* as the type.

A less intensive study has been made of the plant pathogens that have been placed in *Corynebacterium*. It is concluded that *Corynebacterium michiganense* may well belong in that genus, but the inclusion there of *C. fascians* is questionable; *C. flaccumfaciens* and *C. poinsettiae*, however, should not have been placed in it, chiefly because they are motile, with a single flagellum at one pole.

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