BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

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THE GENERIC CLASSIFICATION OF THE SOIL CORYNEBACTERIA

Francis E. Clark*

The transfer by Murray et al. (1) of the genus Corynebacterium from the order Actinomycetales to the Eubacteriales, its separation from the genus Mycobacterium with which it had been closely grouped for many years, and the inclusion within the genus Corynebacterium of a number of species of soil, cellulolytic, and phytopathogenic bacteria previously assigned to other genera, are indicative of the present unsettled taxonomy of the corynebacteria.**

One need look no further than the reference already cited for such statements as "the reports of motile species in this genus present a puzzling problem" and "the relationship of the following soil organism (Bacterium globiforme) is not clear, but it apparently should be placed either in Corynebacterium or in a related genus (e.g., Mycobacterium).

The latter statement focuses one part of the problem especially sharply, inasmuch as in the Bergey Manual (2) the "related genus", after a half century of association, is found not merely in a separate family, but even in a separate order. It is also of interest to note that although Conn contributed Appendix III of Corynebacterium to the Manual, he immediately published elsewhere (3), assigning the appended group of globiform bacteria to the order Actinomycetales.

Several recently published expressions of dissatisfaction with the taxonomy of the soil and plant corynebacteria, the appearance of some alternative schemes of classification, and the accumulation of some additional observations concerning the morphology and physiology of this group of microorganisms have made it appear desirable to review current problems in the taxonomy of the soil corynebacteria and of certain closely related bacteria.


** The term corynebacteria is here used as a convenient designation to include not only species which may be placed in the genus Corynebacterium but also those which may be placed in closely related genera. It may be regarded as substantially equivalent to the family Corynebacteriaceae. Similarly the name mycobacteria includes not only organisms placed in the genus Mycobacterium but also those in other closely related genera of the Mycobacteriaceae.
Has The Genus Corynebacterium Become Unwieldy?
The difficulty of separating corynebacteria of animal origin from gram-positive, pleomorphic bacteria from other sources was apparent to Lehmann and Neumann (4) and to many succeeding writers. The saprophytic corynebacteria that occurred in soil, air, and water were considered clinically unimportant, and they received very little attention from medical bacteriologists. For the most part, soil bacteriologists gave their attention to bacteria accomplishing mineral nutrient transformations believed important in soil productivity. A few workers, however, directed their efforts to a study of the general soil microflora, and in the course of their work, an ever greater variety of microorganisms has been assigned to Corynebacterium.

The work of Jensen (5) played an especially important part in the development of a trend to broaden Corynebacterium to include therein a number of bacteria of soil and plant origin. Following careful study of the morphology and physiology of the small angular rods predominant in soil, Jensen (5) described several of them as new species in the genus Corynebacterium. In addition, he established new combinations in Corynebacterium to accommodate seven species previously assigned to four other genera. The species transferred to Corynebacterium were the following: Aplanobacter insidiosum McCulloch, A. michiganense Erwin F. Smith; Bacterium helvolum (Zimmermann) Lehmann and Neumann, B. nubilum (Frankland) Lehmann and Neumann; Cellulomonas fimii (McBeth and Scales) Bergey et al.; Microbacterium lacticum Orla-Jensen, and M. liquefaciens Orla-Jensen. These several new combinations were made by Jensen because of his firm belief that the bacteria concerned formed a natural and consistent group.

Plant pathologists showed a willingness to follow the suggestion of Jensen (5) that certain phytopathogenic bacteria should be designated as corynebacteria. Skaptason and Burkholder (6) transferred Bacterium sepedonicum Spieckermann and Kotthoff, the organism causing bacterial ring rot of potatoes, to Corynebacterium. Dowson (7) made new combinations in Corynebacterium to accommodate Aplanobacter rathayi Erwin F. Smith, Bacterium flaccumfaciens Hedges, and Phytomonas fascians Tilford. Starr and Pirone (8) described the new species Corynebacterium poinsettiae as the cause of a disease of the common ornamental poinsettia. Murray et al. (1) recognized these several new combinations or species, and in addition, made new combinations to place in Corynebacterium the following: Aplanobacter agropyri O’Gara, Pseudomonas hypertrophicans Stahel, and Pseudomonas tritici Hutchinson.

In addition to their recognition of phytopathogenic corynebacteria and of several saprophytic corynebacteria from soil as members of the
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In 1928, Conn (10) described the species *Bacterium globiforme* as representative of the pleomorphic, gram-variable, slow-growing bacteria in soil. This species subsequently was designated as *Achromobacter globiforme*, order *Eubacteriales*, by Bergey et al. (11). Bacteria undoubtedly identical with the globiform group of Conn were placed in *Mycobacterium* by Krassilnikov (12). Following the work of Jensen (5), the close relationship of the globiform group to *Corynebacterium* became generally recognized. In the sixth edition of Bergey's Manual (2), soil species such as *Corynebacterium helvolum* and *C. tumescens* were placed in *Corynebacterium*, and *Bacterium globiforme* was placed in an appendix to the genus. Recently, Wood (13) has proposed *Corynebacterium globiforme* as a new combination.

It is obvious that *Corynebacterium* has come to include many bacteria formerly found in other genera. Conn (14) and Conn and Dimmick (3) objected to the inclusion of an ever greater variety of organisms in *Corynebacterium*. Murray et al. (1) and Cowan (15) have also admitted dissatisfaction with the present composition of the genus *Corynebacterium*. One criticism commonly raised is based upon the taxonomic principle that a genus is exemplified specifically by its type. Many of the corynebacteria of soil and plant origin differ markedly from *C. diptheriae*. Therefore, it appears unacceptable to many to place in *Corynebacterium* gram-variable, pleomorphic bacteria that possess flagella, or grow well on inorganic sources of nitrogen, or liquefy gelatin or attack cellulose, or even fail to grow at 37°C. It can be argued that the differences between *C. diptheriae* and *Mycobacterium tuberculosis* are no greater.

It is the writer's opinion that with the gradual extension of its generic limits to include a diversity of bacteria, *Corynebacterium* as currently defined has become an undesirable and an unwieldy genus.

**To What Genus Do The Soil Corynebacteria Belong?**

If *Corynebacterium* is rejected as the generic name for the soil diptheroids, the question then arises whether or not there exists any
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genus to which these bacteria properly can be assigned. Conn and Dimmick (3) have proposed that the soil corynebacteria be designated as Arthrobacter. This name was used by Fischer (16) for non-flagellate rod-shaped bacteria producing "arthrospores." No type was described, and the genus cannot be considered to have been validly published prior to 1947. Therefore the genus should be recognized as Arthrobacter Conn and Dimmick, and not as an emendation of Arthrobacter Fischer, as Conn and Dimmick (3) have proposed.

It is believed desirable to remove the soil globiform organisms from Corynebacterium, and Arthrobacter appears to be an acceptable alternative designation. Lochhead (17) has commented on Arthrobacter as follows: "The accommodation of the Bacterium globiforme group in a separate genus has much to commend it, by avoiding undue extension of the commonly held definition of Corynebacterium, while providing for an important group of related forms."

Arthrobacter has been described rather fully by Conn and Dimmick (3), and its complete description need not be repeated here. In my experience, the published description for Arthrobacter, with an exception concerning motility to be discussed shortly, has been found adequate for globiform cultures obtained from widely scattered laboratories in America and abroad, and for many cultures from soil. According to its authors (3), Arthrobacter liquefies gelatin, fails to produce acid from fermentable sugars, and grows well on synthetic media—in contrast to animal diphtheroids. Corynebacterium cultures obtained from veterinary laboratories almost without exception have been differentiable culturally from cultures of Arthrobacter. The finding of occasional intermediate strains that are difficult to classify is by no means limited to the group of bacteria under discussion, nor does it invalidate the use of Arthrobacter for the soil corynebacteria.

Arthrobacter was described by Fischer (16) as nonmotile; Conn and Dimmick (3) failed to mention motility for any of the species they placed in this genus. Of eight cultures of Arthrobacter received from Conn, two have been found to be motile (18). Subsequently Conn in a personal communication commented that he had intentionally omitted motility from the generic description, although he himself had not observed motile strains. Reports of motility for the soil corynebacteria are sufficiently numerous (13, 18, 19, 20, 21) to show that motility must be recognized within this group.

Bisset and Moore (22) also believed the soil corynebacteria sufficiently dissimilar from C. diptheriae to preclude their assignment to the same genus. They proposed the genus Jensenia to include the soil
diptheroids. Unfortunately, they ignored the extended and very valuable earlier work of Conn, Jensen, and others, and apparently failed to undertake studies of any named cultures from other laboratories. In their initial paper (22) on *Jensenia*, the genus was proposed without designation of type species. After this omission was called to their attention, the genus was again described, with *Jensenia canicruria* (23) as the type. This species was described as failing to hydrolyze polysaccharides or proteins, and also, as failing to reduce nitrates. Inasmuch as *Arthrobacter globiformis* is positive in all these respects, it appeared probable that the two species would prove to be entirely dissimilar. This was found to be true on actual examination. *Jensenia canicruria* (N.C.I.B. culture no. 8147) has been found to agree with its published description in its general physiological responses. Morphologically, the culture appears to be a *Nocardia*. *Jensenia* cannot be considered as synonymous with *Arthrobacter*.

The writer has found culture no. 64 of Topping (19) to be identical with *J. canicruria* 8147. Topping (19) placed her culture no. 64 in a group designated "2b, gram-positive, nonmotile, mycelium-forming", and she remarked further: "It is clear that the organisms described in this paper under Group 2b are members of the Jensen's genus *Proactinomyces*!" (i.e., *Nocardia*).

It is probable that Bisset and Moore, in speaking of the "soil diptheroids", either were dealing only with *Nocardia*, or they grouped the non-mycelium-forming and the mycelium-forming soil organisms into a single genus. Any such action appears highly questionable at the present time.

**The Cellulolytic Soil Corynebacteria**

That certain of the soil bacteria capable of attacking cellulose are in fact corynebacteria has been recognized by Jensen (5), Harmsen (24), and Clark (9). Production of cellulase is known among many taxonomic groups; it should be given no greater taxonomic emphasis than other physiological characteristics. If the cellulolytic corynebacteria were entirely similar to *Arthrobacter* except for cellulase activity, it probably would be desirable to group them all into a single genus. Inasmuch as *Cellulomonas* was validly described some twenty years earlier than *Arthrobacter*, it would be necessary to retain *Cellulomonas* as the generic designation for the combined group.

Such consolidation into a single genus does not appear to be necessary. *Arthrobacter* and *Cellulomonas* differ in certain respects other than their cellulolytic activity. All species of *Cellulomonas* available for study have produced distinct acidity in dextrose nutrient broth, they have shown scant growth on synthetic media, and they have been unable to hydrolyze urea or uric acid. In view of these differences, it is
believed that both genera *Cellulomonas* and *Arthrobacter* should be maintained.

Inasmuch as *Cellulomonas* initially was described to include a number of cellulolytic bacteria, some of which have since been removed to other genera, the generic description given in Bergey's Manual (2) no longer is entirely satisfactory: "Small rods with rounded ends, non-spore-forming, motile with peritrichous flagella, occurring in soil and having the property of digesting cellulose. Growth on ordinary culture media often not vigorous. Gram-negative."

The following emendation is proposed: *Cellulomonas* Bergey et al., emend. Small pleomorphic rods, straight to angular or slightly curved, with occasional beaded, clubbed, branched, or coccoid cells, the number of such cells depending on the age and condition of the subculture. Motile with one or few peritrichous flagella; some species are nonmotile. If only a single flagellum is present, it usually is polar. Gram-reaction variable. Growth on ordinary culture media often not vigorous; otherwise, growth on solid media usually soft and smooth, and in broth, turbid. Yellow pigmentation common; other pigments also occur. Gelatin slowly hydrolyzed. Catalase positive. Acid but no gas from carbohydrates; cellulose commonly attacked. Typically of soil or plant origin.

The type species is *Cellulomonas biazoetea* (Kellerman) Bergey et al.

Heretofore, the genus *Cellulomonas* has been placed in the family *Bacteriaceae*. The emended genus is placed in the family *Corynebacteriaceae*.

**The Phytopathogenic Corynebacteria**

Those workers who have objected to the use of *Corynebacterium* for the soil corynebacteria have also objected to its use for the phytopathogenic corynebacteria. Conn and Dimmick (3), following study of several phytopathogenic species, concluded that, with the possible exception of *Corynebacterium michiganense*, the cultures showed such differences from the type *C. diptheriae* that they clearly belonged elsewhere. More recently, Cowan (15) has proposed that the motile phytopathogens, such as *C. flaccumfaciens*, should be removed from *Corynebacterium*.

Currently, there appear three alternatives for the generic designation of the phytopathogenic corynebacteria: (a) these bacteria can be retained in *Corynebacterium*; (b) they can be placed in *Cellulomonas*; or (c) they can be established in a new genus in the family *Corynebacteriaceae*. Their gram variability, cell morphology, and cultural
properties preclude their return to such genera as *Bacterium* or *Pseudomonas*, where some of them have at times been placed. The genus *Arthrobacter* as defined by Conn and Dimmick (3) did not include the phytopathogenic corynebacteria. If *Arthrobacter* is defined sufficiently narrowly to eliminate confusion with *Cellulomonas*, then its definition will also exclude the phytopathogenic corynebacteria.

In order to avoid premature establishment of new combinations or of additional names, the first alternative, retention in *Corynebacterium*, is followed for the present. Nevertheless it is believed that the allocation of the phytopathogenic corynebacteria to *Cellulomonas* would be preferable to their placement in *Corynebacterium*. In general they show greater similarity to *Cellulomonas biazotea* than to *Corynebacterium diphtheriae*. Attention has been called recently to the similarity in the motility and flagellation of phytopathogenic and cellulolytic corynebacteria (18).

It is also possible that the phytopathogenic corynebacteria should be recognized in a separate genus, distinguished from *Cellulomonas* by their phytopathogenicity and by their lack of cellulase activity. In this connection possibly *Corynebacterium*, at one time suggested by Orla-Jensen (25) as a name for the corynebacteria, could be suitably validated and employed. Inasmuch as action in accord either with this or with the second alternative given above appears desirable, it is to be hoped that some such action will be taken by competent students of this group, in order that the genus *Corynebacterium* can be divested of the phytopathogenic bacteria which differ so markedly from *C. diphtheriae*.

**To What Family And Order Do The Corynebacteria Belong?**

*Corynebacterium* Lehmann and Neumann 1896, together with *Mycobacterium* and *Oospora*, were placed by its authors (26) in the *Hyphomycetes*, their fourth major grouping of microorganisms. In the second edition of the Lehmann and Neumann Manual (27), *Oospora* was replaced by *Actinomyces*, and *Hyphomycetes* by *Actinomycetes*. In the seventh edition (28), *Corynebacterium* and *Mycobacterium* comprised the family *Proactinomycetaceae* in the order *Actinomycetales*.

The extent to which the schemes of other taxonomists, during the period 1896 to 1927, deviated from the proposals of Lehmann and Neumann has been adequately summarized in the introductory pages of Bergey's Manual (2). For the most part, *Corynebacterium* and *Mycobacterium* were included in the same family, and usually in the order *Actinomycetales*. There were relatively few expressions of disagreement. Notably, Winslow et al. (29) recognized six genera in the family *Mycobacteriaceae* in the order *Eubacteriales*. The genera so
grouped were the following: Corynebacterium, Mycobacterium, Nocardia, Fusiformis, Actinomyces, and Leptotrichia. Their proposed placement of these genera in Eubacteriales was not generally accepted in the years immediately following publication.

In agreement with the outline classification of Lehmann and Neumann, Bergey et al. (3) placed the family Mycobacteriaceae, including Corynebacterium and Mycobacterium, in the order Actinomycetales. Four succeeding editions of the Bergey Manual retained this allocation of the genus Corynebacterium. Nor was the taxonomic treatment accorded to the genus by Pringsheim (31), Janke (32), or Pribram (33) greatly different.

Recently, and concurrently with the trend to broaden Corynebacterium to include many bacteria formerly allocated to other genera, there again have appeared some suggestions that the genus Corynebacterium should be assigned to the Eubacteriales and not to the Actinomycetales. Kluyver and Van Niel (34) recognized the Mycobacteriaceae as a family of permanently nonmotile rod-shaped bacteria, and proposed to include therein two tribes, the Corynebacterieae and the Mycobacterieae. Stanier and Van Niel (35) also included both Mycobacterium and Corynebacterium in the Eubacteriales. In criticism of the Bergey's Manual (36) they stated that the inclusion of these genera in Actinomycetales leads to confusion, since these forms can so readily be taken for representatives of the Eubacteriales. On the other hand, they admitted that the dividing line between the genera Mycobacterium and Nocardia was a tenuous one.

Although Stanier and Van Niel (35) had included both the mycobacteria and the corynebacteria in Eubacteriales, Breed et al. (2) separated the two genera, placing Corynebacterium in Eubacteriales, and Mycobacterium in Actinomycetales.

Several workers have raised objection to any such assignment and separation of the corynebacteria and the mycobacteria. Conn and Dimmick (3) preferred the classification given in the fifth edition of Bergey's Manual to that proposed in the sixth edition; they regarded the two genera as closely related. They believed that the Mycobacteriaceae should be divided into three genera, namely, Mycobacterium, Corynebacterium, and Arthrobacter.

Bisset and Moore (22) grouped Mycobacterium, Corynebacterium, and Nocardia in the family Mycobacteriaceae, and the genus Actinomycetes and the newly proposed genus Jensenia in the family Actinomycetaceae, with both families in the order Actinomycetales.
Cowan (15) also placed Mycobacterium, Corynebacterium, and Nocardia, as well as Erysipelothrix and Kurthia, in the family Mycobacteriaceae, but he proposed that this family be placed in a separate order, Mycobacteriales. The families Actinomycetaceae and Propionibacteriaceae comprised the order Actinomycetales.

Altogether, it appears from the recent literature that the majority of those who have worked with the corynebacteria oppose any transfer of this group to the Eubacteriales, and in addition oppose any wide separation of the mycobacteria and the corynebacteria. The fact that certain species now grouped with the corynebacteria have in the past been grouped with the eubacteria hardly seems a sufficient argument for placing all corynebacteria in Eubacteriales. Cowan's proposal (15) that the corynebacteria and mycobacteria be placed in a new order Mycobacteriales appears worthy of endorsement. With the currently proposed grouping of the corynebacteria into several genera (Corynebacterium, Arthrobacter, Cellulomonas), the retention of the family Corynebacteriaceae also appears desirable.

Summary

The genus Corynebacterium Lehmann and Neumann (type species Corynebacterium diphtheriae) should be so defined as to exclude many corynebacteria of soil and plant origin that in recent years have been placed in this genus.

That group of species typified by Bacterium globiforme Conn is recognized as constituting the genus Arthrobacter Conn and Dimmick. The following characteristics of the genus Arthrobacter differentiate it from the genus Corynebacterium: growth on synthetic media, hydrolysis of gelatin and casein, slight or no acid production in dextrose nutrient broth (dextrose and several other carbohydrates are utilized), optimum growth temperature 30 to 33°C, sometimes motile.

The genus Jensenia Bisset and Moore, described by its authors to accommodate the soil diphtheroids, has been found closely related to, if not identical with, Nocardia Trevisan. Jensenia is not a synonym of Arthrobacter. The following characteristics of Jensenia differentiate it from Arthrobacter: micro-colonies on agar show an early open mycelium; does not hydrolyze starch or proteins; does not reduce nitrates.

Corynebacterium fimi (McBeth and Scales) Jensen, together with a group of closely related species such as Bacillus bijazoteus Kellerman et al. placed in the genus Cellulomonas by Bergey et al. are retained in Cellulomonas. This genus is emended in re-
cognition of its close relationship to *Corynebacterium*. The type species is *Cellulomonas biazothea* (Kellerman) Bergey et al. The following characteristics of *Cellulomonas* differentiate it from *Arthrobacter*; distinct acid production in dextrose nutrient broth; commonly cellulolytic; scant or no growth in synthetic media.

Several species of phytopathogenic corynebacteria, previously placed in such genera as *Aplanobacter*, *Bacterium*, *Phytomonas*, or *Pseudomonas*, none of which are tenable for this group, currently are left in *Corynebacterium*. Nevertheless it is believed that they should be segregated from *Corynebacterium*. Their actual transfer to a more satisfactory genus is left to those especially familiar with the phytopathogenic bacteria.

The genera *Corynebacterium*, *Arthrobacter*, and *Cellulomonas* are placed in the family *Corynebacteriaceae* and in the order *Mycobacteriales*. Objection is made to inclusion of the *Corynebacteriaceae* in the order *Eubacteriales*.

References

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