ON THE CLASSIFICATION OF ACTINOMYCETES[1]

SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Station, New Brunswick, New Jersey

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The exact position of the actinomycetes or ray-fungi in the kingdom of lower forms of life has attracted considerable attention and has been a subject of much speculation. These organisms have been classified as bacteria, as fungi, and as a special group, either derived from one of the above or giving rise to both. With the accumulated knowledge concerning the morphology of the actinomycetes, it is being recognized more and more that they are an independent group of organisms, which is closely related to the bacteria through some of the constituent forms, but which has adopted a fungus-like form of growth. A brief description of the mode of growth and spore formation of these organisms will suffice to indicate their exact taxonomic position.

Actinomycetes are characterized by the formation of normally branching threads or rods, frequently giving rise to a typical mycelium which is unicellular, especially during the early stages of growth. The hyphae are generally non-septate; under certain special conditions, septa may be observed in some forms. The mycelium is either vegetative and growing in the substrate, or aerial, where a special mycelium is produced above the vegetative growth. Actinomycetes reproduce through special sporulating bodies or from parts of the vegetative mycelium. The spore-bearing hyphae are produced on the mycelium either singly and monopodially, or in broom-like or cluster-like formations, or in verticilliate-like tufts or whorls upon the mycelium. The sporophores vary from long to very short forms. The spores may also

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be produced singly on the tips of side branches or in chains attached directly to the mycelium. The spores are formed either by the fragmentation of the plasma within the spore-bearing aerial mycelium, or by the process of segmentation or division of the mycelium into reproductive cells by means of cross walls, in a manner similar to oidium formation. The sporulating mycelium may be branching or non-branching, straight or spiral shaped. The spores are spherical, cylindrical or oval; they germinate by the formation of 1 to 3 tubes.

It has long been recognized that the actinomycetes, which have been variously described under the generic names *Actinomyces*, *Streptothrix*, *Nocardia*, etc., comprise a great many species, which could be conveniently grouped under several genera or even families. However, until recent years, most classifications of the group into several large divisions have lacked general approval. This is primarily due to the fact that the various systems proposed for classifying these organisms were either based upon their occurrence in nature, pigment formation upon complex organic media, aerobiosis, proteolytic action, colony formation, or cultural characters upon synthetic media. These systems were primarily ecological or physiological in nature, and may be discarded at the present time as insufficient for the purpose of a sound separation of the actinomycetes, except for minor subdivisions and characterization of species and varieties. These characters may be looked upon as too variable to be of sufficient validity for group characterization. Only upon due recognition of the mode of formation of spore-bearing bodies, were more sound systems proposed; these were predominantly morphological in nature or partly morphological and partly physiological.

Without reviewing in detail the various proposals for classifying these organisms, attention will be called to a few of the more recent systems of classification. Orla-Jensen (1909) included in the family Actinomycetes four genera, the nature of which is evident from their names: *Mycomonas*, *Corynemonas*, *Actinomyces* and *Rhizomonas*. While the inclusion of the first three may be justified, the fourth genus comprising the root nodule bacteria had no place in this family. Buchanan (1918) sug-

gested a new order, Actinomycetales, with a single family Actinomycetaceae. This family was divided into four genera: *Actinobacillus, Leptotrichia, Actinomyces* and *Nocardia.* The first two genera may hardly be considered as typical of the group and as having much in common with true actinomycetes, whereas the difference between the last two genera was based on a fictitious assumption that members of the genus *Actinomyces* do not produce any aerial mycelium and are usually parasitic. The genus *Nocardia* was later dropped by the Committee of the S. A. B. Bergey et al. divided the Actinomycetales into two families, the Actinomycetacecae, containing, in addition to the first three genera included by Buchanan, also the genus *Erysipelothrix,* and the Mycobacteriaceae, with 8 genera, comprising such an oddly assorted conglomerate as *Mycobacterium, Cytophaga, Mycoplana, Cellvibrio* and *Cellfalcicula.* Breed, Murray and Hitchens in the new revision of this manual, left among the Actinomycetaceae only three genera: *Leptotrichia, Actinomyces* and *Erysipelothrix,* and among the Mycobacteriaceae the three genera: *Proactinomyces, Corynebacterium* and *Mycobacterium.*

Lehmann and Neumann also divided the order into 2 families, the Proactinomycetaceae with two genera, *Corynebacterium* and *Mycobacterium,* and Actinomycetaceae, with only one genus *Actinomyces.* Pringsheim (1923) separated the order Mycobacteriales from the Eubacteriales, including the genera *Corynebacterium, Mycobacterium* amd *Actinomyces* in the first. Kluyver and van Niel (1936) suggested the removal of the family Mycobacteriaceae from the Actinomycetales altogether.

As to the more specific systems of classification which limit themselves specifically to the group of actinomycetes, the following may be mentioned. The last three of these systems deserve particular consideration.

Krainsky (1914) divided the group into *Macroactinomyces* and *Microactinomyces* on the basis of colony size. Pinoy (1913) and Chalmers and Christophersen (1916) suggested a system based on oxygen tension, the anaerobic forms being designated as *Cohnistreptothrix* and the aerobic forms as *Nocardia.* A similar system was proposed by Langeron (1922), except that the aerobic

forms were classified as *Euactinomyces*. Wollenweber (1921) emphasized the formation of aerial mycelium and listed *Aerothrix* as producing one and *Pionnothrix* as not producing any aerial mycelium. Lignières (1924) suggested three genera: *Actinomyces* for aerobic forms with non-fragmented mycelium; *Brevistreptothrix* for anaerobic forms with segmented filaments; *Actinobacillus* for rod-shaped cells not forming any mycelium. Ørskov (1923) divided the actinomycetes into 3 genera: *Cohnistreptothrix*, with non-septate mycelium, producing readily sporulating aerial hyphae; *Actinomyces*, without spore formation in the aerial mycelium; *Micromonospora*, with single spores borne at the end of branches. Jensen (1931) modified this system by retaining the last genus unchanged, and designating the first genus as *Actinomyces* and the second as *Proactinomyces*. Krassilnikov (1938) accepted the order of *Actinomycetales* of Buchanan, and divided it into two families: *Actinomycetaceae*, with four genera, *Actinomyces*, *Proactinomyces*, *Mycobacterium* and *Mycococcus*, and *Micromonosporaceae*, with one genus *Micromonospora*.

Each of these systems has some favorable characteristics but tends to give undue emphasis to certain particular groups of organisms in which the author happened to be especially interested. A system is proposed here in which an attempt is made to meet the above limitations, to indicate the relationship of the various constituent groups to the true bacteria, and to include forms not considered by the other authors. In this connection, particular attention is called to the fact that, owing to the great variability and abundance of transition forms among the actinomycetes, the term species should be applied more in the sense of "species-groups." However, certain type species may be established, around which the closely related forms may be grouped.

The various genera included among the Actinomycetales form a natural group of microörganisms with a variety of transitional forms. Ørskov (1923) and later Jensen (1932) emphasized the fact that certain mycobacteria are practically indistinguishable from certain proactinomyces; the same was found to be true of the relationship between corynebacteria and other proactinomyces. On the other hand, the close relation between corynebacteria and typical bacteria found among the Coccaceae has

also been emphasized by many workers. The existence of transition forms between species of *Actinomyces* and of *Proactinomyces* and between species of *Actinomyces* and *Micromonospora* can also be readily demonstrated. Organisms of one group may sometimes by a process of dissociation produce variants indistinguishable from one of the other groups. It becomes frequently difficult, therefore, to determine where to place a certain organism of the borderline type.

Order ACTINOMYCETALES Buchanan

Organisms forming elongated, usually filamentous cells, with definite tendency to branching; hyphae not exceeding 1.5μ in diameter, mostly about 1μ or less. Usually producing a characteristic branched mycelium. Multiply by means of special spores, as well as by oidiospores or by conidia. The spores are formed by fragmentation of the plasma within the spore-bearing hyphae, the latter being straight or spiral-shaped; the oidiospores are formed by segmentation, or by simple division of hyphae by means of transverse walls, similar to the formation of oidia among the true fungi. The conidia are produced singly, at the end of special, simple or branching conidiophores. They grow readily on artificial media and form well developed colonies. The surface of the colony may become covered with a special aerial mycelium. Some of the organisms are colorless or white, whereas others form a variety of pigments. They are either saprophytic or parasitic. In relation to temperature, most are mesophilic while some are thermophilic. Certain forms are capable of growing at low oxygen tension.

A. Mycelium, rudimentary or absent.

Family MYCOBACTERIACEAE Chester

Organisms forming only a rudimentary mycelium, if any. Cells in early stages of growth vary in length, are rod-shaped, and sometimes branching. Multiply by division and bud formation. Characterized by a "snapping" or "angular" type of cell division. Bacteria-like colonies, of a slimy or soft consistency with smooth or folded surface. Growth diffuses in liquid media or readily diffusible. Some forms acid-fast. Pathogenic or saprophytic.

I. Acid-fast organisms.....*Mycobacterium* Lehmann and Neumann. Type species—*M. tuberculosis* (Schroeter) L & N.

II. Non-acid-fast organisms.....*Corynebacterium*, Lehmann and Neumann. Type species—*C. diphtheriae* (Flügge) L & N.

Jensen[2] suggested the desirability of including the genus *Mycoplana* Gray and Thornton under the Mycobacteriaceae. However, this may be held in abeyance until more information is obtained concerning these organisms. The same may be said of other genera such as *Propionobacterium* Orla-Jensen, which was shown (van Niel, 1928) to have a close resemblance to the corynebacteria.

B. Mycelium produced, spores formed by segmentation (formation of septa)

Family PROACTINOMYCETACEAE Lehmann and Neumann

The mycelium of proactinomycetes is non-septate during the early stages of growth but later becomes septate and breaks up into short segments, rod-shaped or spherical in shape. The colonies are bacteria-like in nature, smooth, rough or folded, of a soft to a dough-like consistency, sometimes compact and leathery in young stages. They are either colorless or produce various pigments. Most forms do not produce any aerial mycelium; a few produce a limited mycelium, the branches of which also break up into oidiospores or segmentation spores. This family is distinguished from the previous one by the formation of a true mycelium. As compared with the next family, it is characterized by the manner of spore formation.

I. Genus *Cohnistreptothrix* (*sensu* Pinoy, *non* Ørskov). Anaerobic or microaerophilic, usually parasitic; non-acid-fast, non-proteolytic and non-diastatic.
Type species. *C. israeli* (Kruse) Pinoy.

The possibility of dividing this genus into two species, comprising organisms of human origin ("israeli") and those found in cattle has been suggested (Erikson, 1939).

II. Genus *Proactinomyces* Jensen. This genus comprises the obligate aerobes. It can be divided into 2 subgroups on the basis of acid-fastness and action upon complex carbohydrates and proteins (Umbreit, 1939).

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[2] Personal communication.

Type species. *P. agrestis* (Gray and Thornton) Jensen.

Subgroup 1. Partially acid-fast, non-proteolytic, non-diastatic, utilize paraffin. Usually yellow, pink, or orange to orange-red in color.

Type species. *P. asteroides* (Eppinger).

Subgroup 2. Non-acid-fast, diastatic, largely proteolytic, do not utilize paraffin. Yellow, orange to black in color.

Type species. *P. maculatus* (Millard and Burr) Umbreit.

In addition to these two subgroups, which are designated as β-proactinomyces, another group is frequently included among the proactinomyces, namely, the so-called α-types (18). Here belong forms which are characterized by short, unstable mycelium, producing soft, bacteria-like colonies and growing diffusely in liquid media. The only difference between these forms and the coryne- or myco-bacteria is that the former produce a mycelium which rapidly disintegrates. Most of the proactinomyces found in the autochtonous flora of the soil fall within this group (Lochhead, 1939). This group may be tentatively considered as containing transition forms between the mycobacteriaceae and the true proactinomycetes.

C. Mycelium produced, spores formed in aerial mycelium.

Family ACTINOMYCETACEAE Buchanan

Organisms producing a fine, non-septate, well-developed mycelium with branching hyphae. The hyphae as a rule do not break up into segments. These organisms multiply by spores, oidiospores, and pieces of mycelium. They produce compact, leathery colonies on solid media and grow in liquid media in the form of flakes or minute colonies, frequently forming a solid mat on the surface of the medium; no turbidity produced. Gelatin liquefied, with varying degrees of rapidity, with or without pigment production. This group is composed mostly of soil actinomycetes but there are also pathogenic forms.

I. Spores produced in chains on aerial hyphae arranged monopodially or in clusters..... *Actinomyces* Harz.

Type species—*A. albus* (Rossi Doria) Gasperini.[3]

[3] *A. bovis* Harz is usually given as the type species of this genus. However, recent investigations have brought out the fact that this name is a *nomen dubium* (see E. Baldacci, Mycopathologia, May, 1938). The same is true of *A. hominis* Bostroem.

This genus can be divided, on the basis of the structure of sporulating hyphae into five groups:

Subgroup 1. Straight sporulating hyphae, monopodial branching, never producing regular spirals.
Type species—*A. madurae* (Vincent) L & N.
Subgroup 2. Spore-bearing hyphae arranged in clusters.
Type species—*A. viridis* Lombardio-Pelligrino.
Subgroup 3. Spiral formation in aerial mycelium; long, open spirals.
Type species—*A. albus* (Rossi Doria) Gasperini.
Subgroup 4. Spiral formation in aerial mycelium; short, compact spirals.
Type species—*A. halstedii* Waksman and Curtis.
Subgroup 5. Spore-bearing hyphae arranged on mycelium in whorls or tufts.
Type species—*A. reticuli* Waksman and Curtis.

D. Spores produced terminally on branches of vegetative mycelium.

Family MICROMONOSPORACEAE Krassilnikov

Well developed, fine, non-septated mycelium, 0.3–0.6μ in diameter. Grows well into the substrate. Not forming at any time a true aerial mycelium. Multiplies by means of conidia, produced singly at end of special conidiophores, on surface of substrate mycelium. Conidiophores short and either simple, branched or produced in clusters. Strongly proteolytic and diastatic. Comprises mostly saprophytic forms. These organisms occur mostly in manure, aerial dust and soil; many are thermophilic and can grow at 65°C.

I. Genus *Micromonospora* Ørskov.
Type species—*Micromonospora chalceae* (Foulerton) Ørskov.

This genus could be subdivided on the basis of the relations of the organisms to temperature, since it includes a number of thermophilic forms which grow readily at 55°–65°C., and mesophilic forms having their optimum temperature at 30°C. Each of these groups is divided into 3 subgroups, based on the structure of the spore-bearing hyphae. Among the thermophilic forms only representatives of the first group have so far been isolated in pure culture although the existence of the other two groups has definitely been demonstrated in microscopic preparations.

Subgroup 1. Simple spore-bearing hyphae.
Type species—*M. vulgaris* (Tsiklinski) Waksman.
Subgroup 2. Branching spore-bearing hyphae.
Type species—*M. chalceae* (Foulerton) Ørskov.
Subgroup 3. Spore-bearing hyphae in clusters.
Type species—*M. fusca* Jensen.

The available information does not justify as yet the separation of the thermophilic forms into separate species. However, this may have to be done later when more information has accumulated concerning these organisms.

SUMMARY

The following key is suggested for the classification of the order Actinomycetales:

A. Mycelium rudimentary or absent: Family *Mycobacteriaceae* Chester.
  I. Non-motile:
    (a) Acid-fast organisms: *Mycobacterium* L & N.
    (b) Non-acid-fast organisms: *Corynebacterium* L & N.
  II. Motile: *Mycoplana* Gray and Thornton (tentative inclusion).
B. True mycelium produced:
  I. Vegetative mycelium divides by segmentation into bacillary or coccoid elements: Family *Proactinomycetaceae* L & N.
    (a) Anaerobic or microaerophilic, usually parasitic, non-acid-fast: *Cohnistreptothrix* Pinoy.
    (b) Aerobic, partially acid-fast or non-acid-fast: *Proactinomyces* Jensen.
  II. Vegetative mycelium normally remains undivided:
    (a) Multiplication by conidia formed in chains from aerial hyphae: Family *Actinomycetaceae* Buchanan: *Actinomyces* Harz (subgroups 1–5).
    (b) Multiplication by conidia formed terminally and singly on short conidiophores: Family *Micromonosporaceae* Krassilnikov; *Micromonospora* Ørskov (subgroups 1–3).

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