THE CLASSIFICATION AND PHYLOGENETIC
RELATIONSHIPS OF THE ACTINOMYCETALES¹

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SUMMARY. The taxonomic and phylogenetic relationships of members of the order Actinomycetales have been examined. On the basis of cellular and colony morphology, cell wall composition, fermentation products, and certain physiological characteristics, the taxa within the family Actinomycetaceae were divided into two groups. Each group was closely related to members of the family Lactobacillaceae. One group consisted of Actinomyces israelii, A. naeslundii, A. propionicus, Nocardia dentocariosus and Odontomyces viscosus ("hamster organism"). The second group consisted of A. bovis, A. eriksonii, and Lactobacillus bifidus type II (L. parabifidus). This latter organism was re-named Actinomyces parabifidus nov. comb. because its morphological, physiological and biochemical characteristics related it to the members of both groups of the genus Actinomyces. The families Streptomycetaceae and Mycobacteriaceae appeared more closely related to the family Corynebacteriaceae than to the family Actinomycetaceae. The use of certain criteria for classification and determination of phylogenetic relationships was discussed. We have stressed those areas in which necessary information is lacking.

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Although there have been several publications concerned with the classification of the aerobic actinomycetes (Gottlieb 1959-1960; Krasilnikov 1960; Lechevalier, Solotorovsky and McDermont 1961; Davis and Freer 1961; Selvestri, Turri, Hill and Gilardi 1962), only a few have dealt with the anaerobic actinomycetes and their relationships within the order Actinomycetales (Cummins and Harris 1958, 1959; Buchanan and Pine 1962; Cummins 1962; Overman and Pine 1963). Hesseltine (1960) considered both groups but his discussion of taxonomic and evolutionary patterns dealt primarily with the nonpathogenic aerobic actinomycetes. In addition, in recent years several new taxa of actinomycetes have been described (Howell, Murphy, Paul and Stephan 1959; Davis and Freer 1960; Gilmour, Howell and Bibby 1961; Buchanan and Pine 1962; Howell 1963; Georg, Robertstad, Brinkman and Hicklin 1964). This report deals with the order as a whole with emphasis on the anaerobic to facultative organisms. It attempts to incorporate the newly described genera and associated information into a classification scheme that is not only useful but which also reflects their phylogenetic relationships.

Relationship of the actinomycetes to
other groups of the plant kingdom

In his analysis of the comparative characteristics of the actinomycetes, fungi and bacteria, Hesseltine (1960) supported the view that the actinomycetes represented a separate phylogenetic line. Our conclusions, based upon essentially the same considerations but including recent information, is that the actinomycetes should be considered as bacteria and as such should be classified in the class Schizomycetes (Breed 1957), order Actinomycetales. This conclusion is based upon the following considerations:

1. The organisms of this order are all of bacterial size and the internal structures such as the nucleus, membranous organelles, ribosomes, cell membrane, cell wall, and absence of mitochondria would appear to place them without question in the class Schizomycetes. Studies particularly appropriate to this consideration are those of Moore and Chapman (1959) on the growth of a streptomycete, Glauert and Hopwood (1959) and Hopwood and Glauert (1960) on

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Streptomyces coelicolor, Koike and Takeya (1961) on mycobacteria, Edwards and Gordon (1962) on Actinomyces bovis, Gordon and Edwards (1963) on Dermatophilus congolensis, Imaeda and Ogura (1963) on Mycobacterium, and Overman and Pine (1963) on Lactobacillus bifidus and on 4 species of Actinomyces.

2. All the organisms of this order that have been tested are sensitive to antibacterial antibiotics but are resistant to those affecting only the fungi such as griseofulvin, nystatin, amphotericin and candicidin (Lechevalier, Acker, Corke, Haenseler and Waksman 1960; Feingold 1963; Lampen 1963). With increased knowledge of the mechanisms of functions of the antibiotics (Feingold 1963) these differential antibiotic activities are considered to have phylogenetic importance.

3. Cell wall composition, of those actinomycetes tested by Cummins (1962), as compared to recognized bacterial and fungal species (Cummins and Harris 1956) showed a direct relationship between the members of the order Actinomycetales and other bacteria. This relationship is best described by the presence in the cell walls of a combination of amino acids, hexosamines, and sugars (Salton 1962) and by the presence of muramic acid and diaminopimelic acid or lysine. Muramic acid, diaminopimelic acid and lysine are not found in cell walls of the members of the phylum Mychota (Davis 1961). The higher fungi have shown only the presence of sugars and their amino derivatives. All the organisms of the order Actinomycetales are Gram-positive, a further reflection of their cell wall composition (Salton 1962), and consequently, of those tested, most have shown a high degree of sensitivity to penicillin.

4. Finally the infection of actinomycetes by phage strongly suggests the bacterial nature of this group since such infections in the Eumycetes have been limited to a single report. Carvajal (1953) has described industrial fermentations by Streptomyces which became infected with phage. Bradley and Anderson (1958) and more recently Bradley (1964) have reported on the infection of Streptomyces and Nocardia with phage. The morphological description of cells in culture and electron microscopic observations of ultrathin sections of Actinomyces propionicus show most probably the presence of a lysogenic phage (Buchanan and Pine 1962; Overman and Pine 1963).

Although further arguments could be presented, the above considerations appear to be sufficient to retain this group within the Schizomycetes, order Actinomycetales.

The order Actinomycetales in the following classification is a morphological order, i.e., all of the members relate to one another by the ability to form, under the appropriate conditions, filamentous or branched cells and the ability to form mycelial or pseudomycelial colonies. Although one immediately becomes conscious of certain weaknesses in or possible exceptions to this description, other physiological and biochemical properties serve well to maintain the integrity of the order. However, as will be pointed out later, a stronger phylogenetic classification appears to result if branched cells or mycelium formation is not given primary importance.

In order to arrange the various families and member genera into a functional sequence, with reasonable phylogenetic relationships, certain basic premises are made. First, the concept of Oparin (1938) is accepted that evolution proceeded from the lesser to greater enzymatic complexity, that the heterotroph is more simple enzymatically than the autotroph. Secondly, it is assumed that the heterotrophs within the order became more complex as the nutritional demands became simpler, and as the organisms proceed from a strictly anaerobic growth through a facultative one to one which is strictly aerobic. In our classification no direction of the phylogenetic progression is inferred, nor is it of importance in delineating mutual relationships. Recognizing that the loss of an enzyme through mutation is more easily accomplished than the gain of an enzyme, we subscribe to the idea of evolution progressing in a manner of a pair of cones placed apex to apex as described by Pirie (1957). In this the progression from heterotrophy to autotrophy is compared as the progression from the base to the apex of one cone and cellular differentiation and enzymatic loss then progresses from the apex of the second cone to its base.

Enzymatic complexity is intimately connected to morphological complexity. In this regard, the assumption is made that a spherical cell and a rod are of equal complexity, for the mechanisms involved in forming a cell wall in the coccus would not appear to require any fewer enzymes than in the rod (Cole 1962, 1964). The third premise states that the

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bacterial rod is no less complex than a branched or filamentous organism. This is predicated in part on the results of Nickerson and Webb (1956) and Webb (1963), who showed that rod-shaped organisms can be induced to form long filamentous threads with minor element deficiency or folic acid antagonists. It is obvious that in these circumstances, cross wall formation was impaired and that growing tips were maintained at only 2 points at most. The third premise is further supported by L. bifidus. In low concentrations of muramic acid or its precursors this bacterium changes from an essentially rod-shaped organism budding at the ends to one which is multibranched and atypical in its morphology (Glick, Sall, Zulliken and Mudd 1960) and in which growing points originated at various locations along the cell. The formation of spores, particularly those formed on or within specialized structures such as sporangia or conidiophores, would appear to represent a more complex system of enzymatic activity and internal control than the formation of oidia by separation of the cellular components of the hyphae. The fourth and final premise is based upon Cummins and Harris' (1956) and Cummins' (1962) considerations of the phylogenetic significance of cell wall composition. In essence this was formulated by Cummins (1962) as: those groups which have a similar basic skeletal cell wall composition are more closely related phylogenetically than those groups with different basic skeletal cell wall composition.

On the basis of these predications, morphologic criteria, the observations of Austwick (1958), Lechevalier et al. (1961), Cummins (1962), Buchanan and Pine (1962), Cross, Lechevalier and Lechevalier (1963) and Bergey's Manual of Determinative Bacteriology, 7th ed., and using McClung's groups I, II, and III (McClung 1954) as a morphological description of colonial structure only, a new classification is presented along with phylogenetic relationships. Our classification within the Streptomycetaceae is presented only as a device for emphasizing the over-all relationships of anaerobic members to other members of the order. A more complete classification of the aerobic sporulating genera has been given by Lechevalier and Lechevalier (1965).

Order Actinomycetales

Organisms composed of elongated cells that have a definite tendency to branch. Hyphae do not exceed 1.5 μ in

width and are mostly $1.0\ \mu$ or less in diameter.

- I. True mycelium formed on the surface of agar media or under certain conditions in tissues. In culture, branched filaments or budding cells are always formed; aerial mycelium and spores are never formed; anaerobic, facultative, and aerobic; colonies of McClung's (1954) group I. Fermentation of glucose occurs under anaerobic, microaerophilic or aerobic conditions with the production of volatile and non-volatile acids. . . . Family: Actinomycetaceae
- A. Anaerobic to facultative, catalase negative, true mycelium formed but may be transitory. Glucose fermented to produce characteristic amounts of volatile (formic, acetic, or propionic) acids and lactic and succinic acids. . . . Genus: Actinomyces
- B. Facultative, catalase positive. True but transitory mycelium formed. Formic, acetic, lactic and succinic acid formed from glucose.
Genus: "Hamster organism" (Howell, 1964)*
Odontomyces*
- C. Strict aerobe, catalase positive, true mycelium formed. Acetic and lactic acids are major products formed from glucose under aerobic conditions.
Genus: "Nocardia" dentocariosus (Roth and Thurn, 1962)

* A generic and specific name (Odontomyces viscosus) for this "Hamster organism" (Howell 1964) has been submitted by Howell, Jordan, Georg and Pine, 1965 (Sabouraudia, in press).

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- II. True primary mycelium is always formed adherent to the agar and a secondary aerial mycelium more or less perpendicular to the surface of the mycelium may or may not be formed; isolated spores or spores in chains usually formed, but "sporangia" are never formed. Cellular division by transverse wall formation only. Spores nonmotile. Strict aerobes. McClung's (1954) colony groups II and III.

Family: Streptomycetaceae

- A. Conidia formed terminally and singly on short sporophores.

1. Secondary aerial mycelium not formed. No growth between 50 and 65°C.

Genus: Micromonospora

2. Secondary aerial mycelium formed. Optimum growth between 50 and 65°C.

Genus: Thermoactinomyces

- B. Conidia formed in longitudinal pairs on the aerial mycelium.

Genus: Waksmania

- C. Conidia formed singly and in chains on both the substrate and aerial mycelium.

Genus: Micropolyspora

- D. Conidia when formed are in chains on the aerial mycelium only.

1. Mycelium fragmenting to form bacillary or coccoidal elements, secondary aerial mycelium and conidia often absent. . Genus: Nocardia

2. Mycelium nonfragmenting, secondary aerial mycelium and chains of conidia generally formed.

Genus: Streptomyces

III. Mycelium rudimentary or absent. Branched cells may or may not be formed. True mycelium not formed on the surface of agar media but found occasionally in clinical materials. McClung's group I. Strict aerobes. Spores are never formed. . Family: Mycobacteriaceae

A. Cells usually acid-fast. Rod-shaped cells that do not branch under ordinary cultural conditions.
Genus: Mycobacterium

B. Cells nonacid-fast. Cells generally spherical, occurring singly or in clumps. . Genus: Mycococcus

IV. True primary mycelium formed with subsequent fragmentation into coccoidal cellular units. Cellular division by transverse and longitudinal cell wall formation. Secondary aerial mycelium not formed. Motile spores formed by longitudinal and transverse division of hyphal structures not sporangia.
Family: Dermatophilaceae
Genus: Dermatophilus

V. A true mycelium formed; spores formed in sporangia.
Family: Actinoplanaceae

A. Motile spores. Secondary aerial mycelium not formed.
Genus: Actinoplanes

B. Nonmotile spores, secondary aerial mycelium abundant, large, spherical sporangia (7-19 μ).
Genus: Streptosporangium

C. Nonmotile spores, secondary aerial mycelium abundant, small club-shaped sporangia with single row of spores produced on both substrate and aerial mycelium. Sporangia 2-9 μ x 1.5-3.6 μ .
Genus: Microellobosporia

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It is seen that contrary to the suggestion of Lechevalier et al. (1961) the family Streptomycetaceae is retained. Although these workers argued for its abolition, an analysis of the physiological and morphological characteristics of certain members of the order show that a very useful and logical division can be had by retaining the two families, Actinomycetaceae and Streptomycetaceae. We have placed the genus Nocardia in the family Streptomycetaceae since in our opinion the genus is most closely related to other members of the family Streptomycetaceae as we have listed them. This conclusion has been supported by the recent publication of Lechevalier and Lechevalier (1965) who, on the basis of morphology and cell wall composition, have also removed the genus Nocardia from the Actinomycetaceae. These authors have placed Nocardia in a new family Nocardiaceae, with other members of the "euactinomycetes." The latter group contains all the aerobic spore-forming mycelial organisms having diaminopimelic acid as a cell wall constituent. Both families as we have given them could contain anaerobic, facultative, or aerobic members forming branched cells, or a transient mycelium, but only the family Streptomycetaceae would have within it those members that formed a true permanent mycelium and those that formed spores on or within specialized structures. However, at present as proposed, the family Streptomycetaceae has only aerobic forms. In regard to the formation of spores, it is still possible that two potential members of the family Actinomycetaceae form spores, or cysts, these being Micromonospora (Hungate 1945) and Arthrobacter as suggested by the work of Cummins and Harris (1959). Further studies are required, however, before the position of these two can be determined.

As conceived by us the family Actinomycetaceae would contain only those members (with two exceptions) that have lysine in their cell walls as opposed to diaminopimelic acid (DAP)—DAP is found in the members of the family Streptomycetaceae (Hoare and Work 1957; Antia, Hoare and Work 1957). The strength of family differentiation based on the presence of one or the other of the amino acids, lysine and DAP, lies in their relationship to metabolic evolution as suggested by Davis (1961), on the fact that all bacteria have shown so far only one or the other of these in the cell wall, on the phylogenetic implications of cell wall composition as discussed by Cummins (1962), and finally by the phylogenetic

relationships within the various groups as will be discussed below. Consequently A. propionicus and the members of the genus Bacterionema would appear to be intermediary in their relationships within the groups. B. matruchotii is facultative to aerobic, catalase positive, forms true mycelium with whip-like filaments terminating in a bacterial-like rod; under limited aerobic conditions growth is poor and propionic and lactic acids are formed from glucose. As discussed below, on the morphological basis, B. matruchotii would best be classified in the family Streptomycetaceae but it does not form aerial mycelium. It does, however, form a terminal structure resembling a promordial "spore." On the biochemical basis it is closely related to the Corynebacteriaceae.

Table 1 presents a summary of the physiological data that served to reveal relationships among various groups. The ideas presented are based upon cell wall analyses, aerobic or anaerobic physiology with the presence of catalase being considered as a link to an aerobic metabolism and the fermentative characteristics of the group. Figure 1 summarizes the data of Table 1, relating the various groups in a rough progression from an anaerobic fermentative metabolism and rod-shaped form to those having an aerobic physiology and mycelium with spores.

In Figure 1 it is seen that the eight organisms listed in the Actinomycetaceae are either anaerobic or facultative, except "Nocardia" dentocariosus which is aerobic. A. parabifidus, A. bovis and A. eriksonii are considered to be related to each other on the basis of fermentation patterns (Pine and Howell 1956; Pine and Boone 1962), ultrafine structure (Overman and Pine 1963) and cell wall composition (Cummins, Glendenning and Harris 1957; Georg, Robertstad, Brinkman and Hicklin 1964). They appear related to L. casei primarily on the basis of cell wall composition. On the basis of physiology and morphology, it would be difficult to assess which is more "complex." However, a second group composed of A. israelii, A. naeslundii, Odontomyces viscosus and "Nocardia" dentocariosus (Roth 1957) shows a definite progression from anaerobic, catalase negative organisms to facultative or aerobic, catalase positive organisms. Concomitantly, the formation of transient mycelium or branched cells by these organisms appears to decrease. Aerial mycelium is never formed and all belong to McClung's group I. All of the species shown in the family Actinomy-

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cetaceae have lysine and galactose as common cell wall constituents with the exception of A. propionicus which has diaminopimelic acid. Because of its facultative metabolism, its mycelial nature, its failure to form catalase, and its pathogenicity for laboratory animals, this species was classified in the genus Actinomyces (Buchanan and Pine, 1962) but it is seen that the presence of diaminopimelic acid in its cell wall relate it to Propionibacterium or B. matruchotii and Nocardia. The members of the group A. propionicus, Propionibacterium, Corynebacterium and B. matruchotii are related by their ability to ferment glucose with the formation of propionic acid either anaerobically or under microaerophilic conditions. A progression exists to the more complex aerobic metabolism since certain members of the genera Corynebacterium and Bacterionema are facultative or aerobes. Cummins (1962) showed that the members of group Nocardia, Micromonospora and Streptomyces have a cell wall containing diaminopimelic acid, but of these, Nocardia strains contain galactose whereas Streptomyces and Micromonospora lack neutral sugars. Of the organisms tested by Becher, Lechevalier, Gordon and Lechevalier, (1964) all the strains of Nocardia had mesodiaminopimelic acid, whereas the strains of Streptomyces had the LL isomer. The relationship of the Nocardia strains to Mycobacterium is shown by the presence of galactose and diaminopimelic acid in the cell wall of the Mycobacterium species and perhaps by the acid-fast characteristics of certain members of the genus Nocardia.

It is evident from Figure 1 that a major problem still to be resolved is a clear description and classification of L. bifidus. There are obviously two groups of organisms included within this species, L. bifidus Type I and L. bifidus Type II (L. parabifidus), (Weiss and Rettger 1938a, b). Both groups and the pertinent references were described in the 6th edition of Bergey's Manual of Determinative Bacteriology (1947). Unfortunately, Type II, that group with which we are most concerned, was deleted in the 7th edition of the Manual. Evaluation of pertinent references given in both editions plus the additional information from the publications of Puntoni (1937), Negroni and Fischer (1944), Norris Flanders, Tomarelli and György (1950), Frank and Skinner (1954), Hayward, Hale and Bissett (1955), Pine and Howell (1956) and Cummins, Glendenning and Harris (1957) leads

to the description of this second group of organisms. Of these references those of Puntoni (1937), Weiss and Rettger (1938a, b), Norris et al. (1950) and Pine and Howell (1956) are used primarily for the description of this taxon which is here placed in the genus Actinomyces.

Actinomyces parabifidus (Weiss and Rettger, 1938) comb. nov. (Bacterium bifidum Orla-Jensen, 1919, 192; Bacteroides bifidus (Group 2) Eggerth, 1935, 295; Lactobacillus bifidus II or Lactobacillus parabifidus Weiss and Rettger, 1938, 115; Actinobacterium bifidum Puntoni, 1937, 157; Cohnistreptothrix bifidus Negroni and Fischer, 1944, 313.)

Habitat: Intestinal tracts of babies, adults, human appendices and fowl feces.

Oxygen requirements: Strict anaerobe, requiring carbon dioxide for growth, but capable of giving limited aerobic growth in shake culture for one transfer. Dies on continued aerobic transfer.

Cell morphology: Bifurcate branching generally occurring at ends of cell with occasional branches in center. Club-shaped cells and atypical bulbous cells formed, cellular morphology strongly influenced by medium. Nonmotile, Gram-positive turning Gram-negative in old cultures.

Colony characteristics: Colonies circular, convex, border entire, white to buff colored, opaque, with smooth to mucoid surface. Colonies soft and cells disassociate readily but young microcolonies may show varying degrees of pseudomycelium formation.

Cultural properties: Optimum temperature 37°C, optimal pH for growth between pH 5.0 and 7.3 with essentially no growth below 5.5.

Physiological properties: Catalase negative, H₂S not formed, nitrates not reduced, gelatin not liquefied, indol not formed, milk acidified and often coagulated.

Fermentation of sugars: Mannitol, glycerol, rhamnose, dulcitol, inositol, amygdalin are not fermented. Acid but no gas formed from glucose, fructose, galactose, sucrose, inulin, raffinose, trehalose, dextrin, arabinose, melizitose, xylose, are usually fermented. Mannose is not fermented (Weiss and Rettger) or poorly fermented (Negroni and Fischer).

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Products of fermentation: Carbon dioxide is not formed, glucose is fermented to form formic, acetic, succinic and lactic acids, of which the acetic acid constitutes 45 to 55% of the glucose fermented and lactic acid 35 to 50% (Pine and Howell). The lactic acid formed is dextro-rotary (L (+)) (Orla-Jensen; Negroni and Fischer; Norris et al.).

Pathogenicity: Nonpathogenic for man or laboratory animals.

It is of interest to note the consideration of Weiss and Rettger (1938) regarding the taxonomic disposition of L. bifidus type II.

"Some may question the wisdom of placing type II in the Lactobacillus genus. There should, however, be no doubt about type I being included among the lactobacilli, because of the very close relationship to L. acidophilus. While type II has many points in common with type I, there are several striking differences. Furthermore, there appears to be no other genus into which this organism can be made to fit. If the lactobacilli are defined as Gram-positive, nonsporing rods which produce relatively large amounts of volatile acid, much of it lactic, from carbohydrates, type II should be conceded a place in this genus."

However, with the findings of Pine and Howell (1956) and Pine, Howell and Watson (1950) that there is indeed a genus which fits type II in regard to morphology, sensitivity to low pH, requirement of carbon dioxide for growth, fermentation products, and the formation of L (+) lactic acid, there is little question as to the validity of its position in the genus Actinomyces. This relationship is emphasized further by the similarity of the cell wall composition of L. bifidus (A. parabifidus) and A. bovis (Cummins, Glendenning and Harris 1957).

A second problem to be solved is the generic name of "Nocardia" dentocariosis for it was recognized by Davis and Freer (1960) and reemphasized here that their organism* is more closely related to the Actinomyces on basis of cell

* N. salivae is considered by Howell and Jordon (1963) and by us to be N. dentocariosus (Onisi) Roth.

wall analyses. It appears to be sufficiently different from Odontomyces viscosus to warrant differentiation from this organism.

The antigenic studies of Cummins (1962) augment and reinforce the conclusions of Cummins and Harris (1958) based on cell wall composition. This might be expected in view of the fact that purified cell walls were used as antigens and that the results obtained reflect their differences in chemical composition. Their results showed a clear relationship among the corynebacteria, mycobacteria, and nocardias. However, strains of Actinomyces and C. pyogenes formed distinct and independent groups. More recently Kwapinski (1964) has presented the results of studies of the antigenic composition of cytoplasmic proteins of strains of Mycobacterium, Corynebacterium, Actinomyces, Nocardia and Streptomyces. On the basis of the precipitation bands observed, the relationships were complex but they would appear to place M. smegmatis in a central position relating Corynebacterium to Actinomyces on one side and Nocardia to Streptomyces on the other. These antigenic relationships are complex and relate to the source of the antigen within the cell. Since such studies as yet have only served to support the divisions made on the basis of other criteria; neither they nor such results obtained with phage cross infection (Manion, Bradley, Zinneman and Hall 1964) have been included in Table 1. But it is obvious that their results as well as those of others (King and Meyer 1962; Georg, Robertstad and Brinkman 1964) need more critical evaluation and assimilation.

Other conclusions presented here reiterate those of Cummins (1958), which were based primarily on cell wall composition. The data in Table 1 show the areas where additional taxonomic studies are required. These lie in the area of cell wall and fermentative analyses and definition of basic metabolism of the various members. Serological and phage studies would probably aid in unmasking more hidden inter-special and generic relationships. At present it would appear that N. dentocariosus is more closely related to the genus Actinomyces than Nocardia; however, these genera would be inappropriate for its classification. The families Propionibacteriaceae and Corynebacteriaceae appear to be more intimately connected to the genus Bacterionema and the family Streptomycetaceae than to the Actinomycetaceae.

Table 1. Biochemical relationships within the orders Actinomycetales and Eubacteriales.

	Cell Wall Constituents						Catalase reaction	Metabolism	Fermentation Products***					
	lysine +, glutamic acid +, alanine +		galactose +				CO ₂	formic acid	acetic acid	propionic acid	lactic acid	succinic acid		
	aspartic acid	glycine	glucose	arab- methyl		mannose								
				inose	sugar									
<u>Lactobacillus casei</u>	+	0	+	0	+	+	0	anaerobe	0	0	0	0	+	0
<u>L. acidophilus</u> *	0	0	+	0	0	0	0	anaerobe	0	+	+	0	+	0
<u>Actinomyces israelii</u>	0	0	0	0	0	0	0	anaerobe	0	+	+	0	+	+
<u>A. naeslundii</u>	0	0	+	0	+	+	0	facultative	0	+	+	0	+	+
<u>Odontomyces viscosus</u>	0	0	+	0	+	+	+	facultative	0	+	+	0	+	+
<u>Nocardia dentocariosus</u>	0	0	0	0	0	0	+	aerobe	+	0	+	0	+	+
<u>A. eriksonii</u>	+	0	+	0	0	0	0	anaerobe	0	+	+	0	+	+
<u>A. bovis</u> *	+	0	+	0	+	+,0	0	anaerobe	0	+	+	0	+	+
<u>A. parabifidus</u>	+	+	+	0	+	+	0	anaerobe	0	±	+	0	+	±
diaminopimelic acid +, glutamic acid +, alanine +														
<u>A. propionicus</u>	+	0	+	0	0	0	0	facultative	+	±	+	+	+	+
<u>Propionibacterium</u>	0	0	0, +	0	0, +	0	+	facultative anaerobe	+	0	+	+	+	+
<u>Corynebacterium</u> *	0	0	0	+	0	0	+	facultative aerobe	+	0	+	+	+	+
<u>Bacterionema matruchotii</u> (L. dentium)	0	0	+	0	0	0	+	facultative aerobe	+	+	+	+	+	+
<u>Nocardia</u>	0	0	0	+	0	0	+	aerobe						
<u>Mycobacterium</u>	0	0	0	+	0	0	+	aerobe						
no galactose +														
<u>Micromonospora</u>	0	+	0	0	0	0	+	aerobe						
<u>Streptomyces</u>	0	+	0	0	0	0	+	aerobe						
<u>N. pelletieri</u>	0	+	0	0	0	0	+	aerobe						

* L. acidophilus and some strains of A. bovis do not have galactose.

** Exceptions: C. pyogenes and C. hemolyticum contain rhamnose and lysine, but no arabinose, galactose or DAP.

*** Products were obtained anaerobically except for N. dentocariosus and B. matruchotii.

Figure 1. Phylogenetic relationships among members of Actinomycetales and Eubacteriales.

The taxonomic position of the genus Bacterionema is not clear, but it is apparently closely related to the family Corynebacteriaceae on the basis of its fermentative products and cell wall composition, although on a morphological basis it could well belong in the Streptomycetaceae. On the other hand, A. propionicus, for reasons stated previously, could remain in the family Actinomycetaceae, but would fit equally well in the Corynebacteriaceae.

The characteristics emphasized in this presentation may appear to strongly de-emphasize the value of branched cells and transient mycelium formation as a criterion for classification within the family Actinomycetaceae. From the standpoint of phylogenetic relationships and practical microbiology we do not believe that the criterion of "branched cells" should be de-emphasized. It is our opinion that this family should be retained for the purpose of accommodating those organisms that have the ability, at least under certain conditions, to form filamentous or branched cells, but which do not produce aerial mycelium or spores. In those particular cases in which definite branching does not occur or mycelium is not formed, the other criteria, given in Table 1, serve to retain all the distinctive characteristics of the family.

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