



**INSECT  
PATHOLOGY**  
*An Advanced Treatise*

**Volume 2**

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# INSECT PATHOLOGY

*An Advanced Treatise*

*Edited by*

Edward A. Steinhaus

*Division of Invertebrate Pathology  
University of California  
Berkeley, California*

Volume 2



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# *Preface*

Inasmuch as the principles which guided the preparation of Volume 2 of this treatise are identical to those that were applied to Volume 1, the reader is requested to consult the Preface of Volume 1 for an explanation of various editorial matters, and of the arrangement and composition of subject matter. Especially are we anxious that the reader be aware of the liberties afforded the authors in selecting their material and in presenting their own concepts and viewpoints even at the sacrifice of uniformity in style or manner of presentation of the different chapters, and why some omissions and some overlapping of subject matter was considered unavoidable and even desirable.

Again, we wish to express our sincere appreciation and gratitude to the publishers, and to the members of their staff, for having undertaken to publish this treatise, and for their skillful and invaluable help and encouragement in accomplishing the preparation of this volume. Also we wish to thank the authors of this volume, as well as those of Volume 1, for their patient cooperation in tolerating editorial changes. Especially are we grateful to Mr. Gordon A. Marsh who again ably assisted the Editor in the preparation of the subject index, as well as in many other ways.

EDWARD A. STEINHAUS

*April, 1963*

*Berkeley, California*

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# *The Taxonomy of Entomogenous Bacteria*

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## I. INTRODUCTION

It is a human characteristic to name everything, and this includes the bacteria. Bacteria, of course, are not aware of this, so their taxonomy is a struggle between the effort of man for order and the apparent "disorder" of bacteria. A brief consideration of the taxonomy of entomogenous bacteria must begin with common problems of bacterial taxonomy as a whole, which in many respects differ from those known to the entomologist in his systematic study of insects.

### A. Some Specific Traits of Bacterial Taxonomy

No one can deny that from the taxonomic point of view there are great differences between bacteria and insects. Therefore, the taxonomic approach to these two forms of life will and must be different. The entomologist deals primarily with morphological characters, while the microbiologist deals primarily with physiological characters; the entomologist bases his classification upon an individual (a type specimen), the microbiologist upon a population of individuals (a type culture); the insect specimen may be dead (at least its development is fixed), while the microbial population is living and dynamic; since the insect type specimen is dead it is therefore unchangeable, while the living microbial type culture may vary and undergo change. Specificity of methods and incomparability of basic criteria emerge from these differences. As morphological characteristics in microorganisms are few and often change during the development of a microbial population, physiological properties remain the principal sources of differential characteristics. Because of their great variability connected with adaptability of microorganisms, the changes in character of an isolated bacterial strain occur quite frequently. In order to identify a bacterium it must be transferred from its natural environment to absolutely artificial laboratory conditions to which the microorganism will only partly adapt. Since the time required for identification is rather long, the taxonomically determined culture is usually many generations from the initially isolated cell. Therefore, one of the basic but very difficult conditions of identification is standardization.

Considering the great variability and adaptability of bacteria, it is important to determine which bacterial features are constant and which are not. Since to do this is not only complicated but often impossible, modern taxonomy abandons the concept of "key features" and concludes that a species must be defined by a complex and correlation of all properties, so it is not important if a particular feature is missing or atypical (for details see Sneath, 1957a). This method has many practical advantages, but it is difficult to follow it in establishing determinative keys. In keys it is necessary to make separations on the basis of several features, a procedure which is at variance with the "complex" conception of the definition of a species. Thus, devoid of the actual descriptions of the species, "these keys, based as they are on inadequate data, do not simplify taxonomy and in some cases can increase confusion by making possible an apparently legitimate diagnosis from an improbable combination of characters" (Cowan, 1956a). The "complex" conception of a species better corresponds to the determinative tables, but so far they have been elaborated satisfactorily only for several groups. For this reason the only

reliable, but very uneconomic, way of determination is to proceed from one description to the other.

Another, and the most basic, difficulty is that in taxonomy of bacteria there is as yet no taxonomic "system." Present manuals are really only catalogues of descriptions. Grouping of species and strains is usually made in a mechanical way and by comparing the properties without revealing their relationships and without establishing any unifying line based on phylogeny or other fundamentals. Thus our present "systems" only outwardly have a logical construction, often built up merely mechanically according to those principles that proved appropriate in the taxonomy of higher organisms, and the suitability of which, for bacterial taxonomy, is still doubtful. It cannot be denied that between the systems of all organisms there must be a certain formal unity, but on the other hand this uniformity cannot be promoted to a "method." At present, this disproportion occurs primarily among the bacteria. Owing to this fact there exist some basically different schemes for the taxonomy of certain bacterial groups, e.g., for Enterobacteriaceae Rahn (see Breed *et al.*, 1957, and the system suggested in Report of the Enterobacteriaceae Subcommittee, 1958). It is, however, possible to make a formal compromise which basically concerns the nomenclature (Cowan, 1956c), but this solution is only temporary and provisional. I am not saying that similar difficulties do not exist with other groups of organisms or that all other systems of classifying organisms are perfect. I only wish to point out that in evaluating bacterial taxonomy our judgment must be very relative, and that frequent contradictions in results may be caused by different approaches to the matter.

#### **B. The Meaning of Taxonomy in Relation to Entomogenous Bacteria**

From the words themselves it is obvious that taxonomy of entomogenous bacteria treats the classification of those bacteria which occur in insects. The term "entomogenous" should indicate the specificity of the subject under consideration. The term is quite clear from a verbal point of view, but it is difficult to define its validity from the standpoint of nature. Only to a limited extent is it possible to make the analogy with, for example, human bacteria or soil bacteria. Bacteria are defined according to the ecology of their occurrence. Thus the criterion is not only the possibility that a bacterium will occur in a certain place, but also the frequency with which it does so, and the biological relation of the organism to its particular environment. By using these criteria in the case of bacteria associated with man it is possible to separate that part of bacterial life which has a relation to man from others and then consider it to be medically significant.

With insects it is more difficult. Man is only one species, ecologically and biologically definitely formed, but insects number many thousands of species which, as a whole, are ecologically unlimited. Therefore, it would seem that it is not possible to speak about "entomogenous bacteria" because the ecological extension of insect species and the ecological distribution of microorganisms may entirely overlap. I am of the opinion that at present the term "entomogenous bacteria" must be understood in two ways, in a broad and in a narrow sense. Under the broad concept we must consider as entomogenous all bacteria other than those we know to be closely bound to other organisms. In the narrow sense entomogenous concerns those bacteria the frequency of which is high in certain insect species and which are in some way closely associated with the insect. It is evident that the term "entomogenous" is a purely ecological and not a taxonomic one. Taxonomy of entomogenous bacteria is only an applied part of the general taxonomy of bacteria. It is a common mistake to suppose that insects have special kinds of bacteria. The only consequence of such a conception has been to bring confusion to the taxonomy of bacteria, which, of course, can be said about any taxonomic conception indiscriminately mixed up with ecological criteria.

## II. OUTLINE OF THE HISTORY OF THE TAXONOMY OF ENTOMOGENOUS BACTERIA

The reader may learn about the history of the taxonomy of bacteria from Bergey's Manual (Bergey *et al.*, 1923; Breed *et al.*, 1957) and from other basic taxonomic compendia (Wilson and Miles, 1946; Krassilnikov, 1949; and others). Generally it can be said that the taxonomy of bacteria began to develop about the year 1885 and that its beginning was characterized by many systems arising from divergent points of view. This situation is very well described by Wilson and Miles (1946, Vol 1, p. 310), who say that the system of classifying bacteria "has developed rather as a result of luck than of cunning." This is true not only for the classification of bacteria, but for that of higher plants and animals as well. The first botanical and zoological systems also failed because a workable classification is very difficult to achieve. Even though there is no published work treating the history of the taxonomy of entomogenous bacteria, it is not the aim of this chapter to do so in detail. The pioneer works of Masera (1936) and Steinhaus (1946a, b) were not intended to be taxonomic treatises and hence present only an organized list of bacterial species mentioned in the literature in connection with insects. The hypothesis that insects have special bacteria (apart from those living in mycetozoa) has resulted throughout the literature in long series of names of species defined by inadequate descriptions, many of which were rather

ill defined even for their time. Moreover, it is possible that some authors did not work with pure cultures. This period lasted until about 1930, and then the interest in bacterial taxonomy somewhat slackened because of the rapid rise of interest in bacterial physiology and biochemistry.

Instead of considering the major taxonomic works chronologically, it is perhaps better to consider them according to their quality, wherein the methods used and the variety of features in the description of bacteria play an important role. Thus in the case of bacteria whose importance was great at the time (e.g., certain bacteria in medicine), the descriptions were constantly supplemented along with the development of identification methods, so that their present descriptions are more accurate than in the case of bacteria lacking this continuity. But even here we can find exceptions—e.g., the well-known *Aerobacter aerogenes* (Kruse) Biejerinck, which still presents a problem (Cowan, 1956b). Successive supplementation of the description of a bacterial species is one of the most important factors for present correct classification. If a poorly described microorganism does not produce any characteristic symptoms in its insect host, or no characteristic properties have been described to distinguish it from similar microbes, it is very difficult to rediscover it and to define it more accurately on the basis of its original description.

An instructive example can be seen with *Bacillus thuringiensis* Berliner or *Bacillus noctuarum* White. Inasmuch as the first of these is—in addition to its selective pathogenicity and associated strictly defined symptoms—characterized by the production of a typical crystalline parasporal body, it was possible to isolate readily identifiable new strains and thus considerably supplement the old descriptions. For this reason we can now recognize with 100 percent certainty the bacillus with which Berliner worked. On the other hand, the new definition (Lysenko, 1958b) of *B. noctuarum* is based on the comparison of newly isolated strains with the old, rather inadequate, description which is indistinguishable from descriptions (modern or old) of a number of other bacteria. If we consider, in addition, that it is an organism belonging to a group which is subject to a great deal of variation, we can see that in this case the probability of it being the same organism as that originally described is less than in the case of *B. thuringiensis*.

Thus there arises the question of what to do with old and ill-defined descriptions. The answer is very difficult. I presume that there are basically only two ways of solving the problem. Either such species should be declared *nomina dubia*, or a new taxonomic status should be found in order to ascertain as precisely as possible to which well-defined bacterial species they correspond, and in agreement with the nomenclatorial code (Buchanan *et al.*, 1958, Rule 24) to carry out synonymity or redescription

if necessary. In my opinion the latter is the most suitable solution because it not only solves the question of taxonomic classification of microorganisms, but also corresponds to the present trend in taxonomy of bacteria—the simplified system. In any event, we cannot avoid a considerable amount of subjectiveness. This point may be a topic for academic discussion, but it does not help the practical needs of taxonomy. From the viewpoint of insect pathology it is more important to bring some order into bacterial taxonomy, even at the cost of being wrong, than to grope our way in a chaos of old names for which we have no valid culture types. To do so would be about the same as using words having no definite meanings.

### III. POSSIBLE CRITERIA TO BE USED IN TAXONOMY

Deeper understanding of the life of bacteria has gone hand in hand with the utilization of this knowledge for the classification of these organisms. Because of the small size of bacteria, reliable morphological distinctions are difficult; thus taxonomy is perforce based on physiological, serological, and, recently, genetic properties (see *Principles of Microbial Classification*, 1954). These features are not absolutely equivalent in value because, besides properties that are considerably well-defined biochemically (e.g., production of urease, VP-MR tests, utilization of hydrocarbons, etc.), we use characteristics the mechanisms of which are very inadequately known (e.g., morphology of colonies, pathogenicity, serological reactions).

There is no uniformity of criteria for classification. Cowan (1959) distinguishes the following main groups of bacterial properties: enzymes and chemical make-up, morphology and staining reactions, serological properties, nutritional requirements, pathogenicity, habitat, and genetic features. To make it simple I shall divide the differentiating criteria into three main groups: cultural properties, pathogenicity, and ecology. I shall not discuss the first group in detail because from the point of view of an insect pathologist it does not vary from common taxonomy, but the other two groups are much more interesting and have a direct connection with an insect pathologist's activity.

#### A. Cultural Properties

As cultural properties we often include morphological and physiological properties, as well as growth and biochemical properties, but in some cases it is difficult to say to which group the property belongs.

A great number of morphological characters are used for systematics, but opinions differ as to their direct utilization for constructing a taxonomic system. As an example one might mention the division of bacteria

according to the distribution of flagella into polar and peritrichous arrangements. This feature was used in Bergey's Manual (Breed *et al.*, 1957) to distinguish bacteria on the level of orders. But some of the bacteria concerned may occur either without flagella or may be "O" forms. The question is where to place these bacteria. This is so with other morphological features, whether it is the shape of cells, the formation of capsules, or other features. It is now clear that morphological characters can be used for classification only when correlated with physiological or serological features.

The suitability of using physiological features for bacterial taxonomy is very well shown from the work of Clarke (1954), and the reader will find many modern tests in papers by Sneath (1956), Rhodes (1959), Ly-senko (1961), and others and in a report of the Enterobacteriaceae Subcommittee (1958). Biochemically well-known features are preferred, since these are closely connected with the development of the physiology of bacteria. It is necessary to realize, however, that for the biochemist, the bacterium is only a model for studying the actual object of his interest, i.e., certain metabolic pathways, whereas for the taxonomist it is the reverse.

Even if biochemical and physiological properties of bacteria are the main basis for the classification of microorganisms, with respect to standardization it is necessary that each property should be identified with the method used, because different modifications of the same method do not always yield the same results. If a certain property is to be used in taxonomy it must be tested in such a way that this test can be carried out by any diagnostic laboratory, because the value of this test depends also on its wide-scale utilization.

## **B. Pathogenicity**

From strictly practical considerations, pathogenicity has often been used as a criterion for separating microorganisms taxonomically. This division may be practically necessary, but it is unilateral. Pathogenicity is not only a property of the bacterium itself, but is the result of the interrelation of the microorganism and its host. This relation is very complicated and multilateral, influenced by the variability and adaptability of the bacterium and also by protective reactions of the host. A symposium was devoted to this question (Howie and O'Hea, 1955), and its results proved that, apart from a few exceptions, we do not know why some bacteria are pathogenic and others are not. A classical example from medical microbiology is *Bacillus anthracis* Cohn and *Bacillus cereus* Frankland and Frankland; for the insect pathologist such an example is *Bacillus thuringiensis* Berliner and again *B. cereus*.

So there is a considerable difference in views of pathogenicity as a property of the bacterium. For the insect pathologist this property is of primary importance, but for the taxonomist it is only one of many properties, and its value may be no greater than is that of another. A number of schemes have been suggested for the actual classification of different types of pathogenicity (Weiser and Lysenko, 1956; Steinhaus, 1959; Lysenko, 1959a; Bucher, 1960; and others). In this paper I shall not analyze their advantages and shortcomings, but shall demonstrate with one example how some of them might be used to aid in the classification of bacteria.

Lysenko (1959a) divides pathogenic bacteria into only pathogenic and facultative (or conditioned) pathogens, while Bucher (1960) distinguishes obligate pathogens, crystalliferous sporeformers, facultative pathogens, and potential pathogens. Lysenko's division is based on the method of penetration of bacteria through the gut wall (Weiser and Lysenko, 1956), where the peritrophic membrane forms a mechanical barrier which conditioned pathogens are unable to overcome without some stress factor. Nonpathogenic bacteria cannot develop in the hemolymph at all because it does not offer suitable living conditions for them, whereas the obligate pathogens are effective either directly through their toxins or they have invasive abilities. Bucher's division essentially corresponds with the foregoing, but his groups, obligate pathogenic bacteria and crystalliferous sporeformers, equal the "pathogenic bacteria" of Lysenko, and Bucher's potential pathogens are equivalent to Lysenko's "conditioned pathogens." Bucher's group of facultative pathogens is a rather unclear interstage between both main groups, according to the foregoing division. I am of the opinion that for bacterial taxonomy the first division is more suitable, as it is simpler and corresponds more closely to the essence of effectively evaluating characters in taxonomy, i.e., in a positive-negative manner.

It is a matter of convenience which determinative criteria we shall use, but it is essential to base the division on simple and reproducible tests. Of course, we must distinguish between pathogenicity and virulence. The first is an attribute of the species, the latter is only the property of individual cultures (Miles, 1955). From the taxonomic point of view this is a relevant difference because pathogenicity is a general consideration, but virulence is directly tested. It is therefore necessary, but also very difficult, to find a correlation between the properties of a microorganism and pathogenicity, because this correlation in various cases will be different, if the species are also different.

On the basis of cultural studies only, we can consider pathogenicity in *B. thuringiensis*, *B. popilliae* Dutky, and a few other, mostly specialized,

microorganisms. Bucher (1960) mentions the correlation between proteolytic activity of bacteria (which can be identified by the liquefaction of gelatin and the decomposition of casein) and the type of potential pathogenicity. This conclusion is very unilateral, because between proteolytic pseudomonads the LD<sub>50</sub> may vary in doses from 3 to 10<sup>4</sup> bacterial cells per animal; on the other hand strongly proteolytic strains of pseudomonads are not pathogenic for the greater wax moth (*Galleria*) even by injections of 10<sup>4</sup> cells per larva (Lysenko, unpublished results). Obviously there are some other mechanisms involved, although proteolytic activity will be effective in the final phases of pathogenesis.

In conclusion, I may say that from the taxonomic point of view pathogenicity can be used as a taxonomic character, but it must be considered only as one of many properties, and not as a special, preferred taxonomic characteristic.

### C. Ecology

In the introduction (Section I, B) I have mentioned some difficulties caused by the wide ecological distribution of insects. Ecology of bacteria has been studied very little, and this more from the viewpoint of various ecological factors than according to where the microorganisms occurred (cf. Williams and Spicer, 1957; Steinhaus, 1960). The ecology of certain types of microorganisms as a taxonomic criterion can be used nowadays more in a sense of negatively limiting than of positively determining.

As an example, we may consider the Enterobacteriaceae. By comparing the distribution of different species of this family with their pathogenicity for their hosts, we find a certain, continuous series of ecological relations. From bacteria solely pathogenic for man (*Shigella*, *Salmonella*) we pass to those pathogenic for and occurring mainly in other warm-blooded animals (*Salmonella*, *Arizona*), then on to the poikilotherms (*Arizona*), and ending with those pathogenic for insects (*Cloaca*, *Serratia*). For example, *Escherichia coli* (Migula) Castellani and Chalmers occurs only in "domestic" insects, such as flies, and we do not find it frequently in caterpillars of agricultural pests. It is analogous to *Proteus vulgaris* Hauser, isolated only from laboratory-bred insects, in which it causes a disease. On the other hand, species of *Brevibacterium* Breed are very often found in insects (Breed *et al.*, 1957; Lysenko, 1959b).

For ecological study it is, therefore, necessary to have a thorough knowledge of individual groups of bacteria, and for taxonomic purposes it would be important to know the full distribution of individual species, for purposes of their classification, but above all for determining the variability of their properties. For general taxonomy it is necessary to know the common distribution of microorganisms because, for example,

a bacterial strain which seems atypical from the standpoint of medical microbiology may have a high frequency of occurrence in insects. Even if we know very little about ecological groups of some entomogenous bacteria, it is possible to presume that they will be mostly associated with certain food preferences of insects. The more specialized the ecological and food areas of an insect species, the more specific will be its microflora.

In the research, as well as in the isolations made from insects, we must be very careful because animals artificially reared have a richer microflora than animals living freely in nature. The larvae of sawflies examined directly in mountain forests have a practically sterile gut, while the intestinal tract of laboratory-bred larvae contains a considerable number of bacteria. The introduction of *Serratia marcescens* Bizio into a population of these larvae living in forests causes the bacterium to settle in the gut, and it can be found there even after 14 days (Lysenko, unpublished results). For this reason great care must be taken when collecting the material for ecological study, otherwise misleading results may be obtained.

I can draw the conclusion that the ecology of bacterial occurrence and distribution may provide very good characteristics for taxonomy, but this aspect of the matter has been studied very little so far.

#### IV. THE RECENT STATE OF THE TAXONOMY OF ENTOMOGENOUS BACTERIA

The main lines of development in present-day taxonomy can be roughly divided into great and small problems. Of the major problems, the foremost have to do with the matter of principles and with the construction of a system, with the definition of species, with intermediate forms, with the appropriateness of differential criteria and the validity of characters, and with the use of statistical methods and ways of elaborating results. The smaller problem is concerned with the revision of partial results, namely the revision and arrangement of different species and groups of bacteria, utilizing new tests and thereby increasing and accelerating the means of identifying bacteria. Taxonomy of entomogenous bacteria is, from the practical point of view, mainly involved with the latter problems, but especially here more harm than profit can ensue without an adequate knowledge of major problems.

##### A. Some New Approaches

Among the new methods of taxonomic study most attention is drawn to those connected with the analysis of the chemical make-up of bacteria. Infrared spectrophotometry (Riddle *et al.*, 1956) or two-dimension chromatography (Cummins and Harris, 1956) have been used for this purpose. We can expect also that the comparative anatomy of the bacterial cell

will be used. Genetic relationships will certainly influence our ideas of the relationship of different bacterial groups. Of course all these methods, many of which cannot be used nowadays for current routine identifications, are really only the initial attempts to find new possibilities.

An old, and as yet unsolved, problem in taxonomy of bacteria is the definition of the basic unit, the species (Waksman, 1957), and the development of the method by which a taxonomic system of bacteria (summarized by Cowan, 1954; Sneath, 1957a) is constructed. Whether we like it or not, it is clear that the system selected must be hierarchic and must be based on binominal nomenclature. Nomenclature may be a matter of convention (i.e., of agreement or convenience), and in some cases such convention may be the extent of the definition of a particular species; however, convention cannot be used as a basis for constructing the system. We cannot exclude the term species just because we do not know how to define it. In any case if the term were abandoned, we would have to replace it with another one. I suppose that most misunderstandings in the definitions arise from the fact that we mix the philosophical aspects with those of pure application, as it would be, for example, in philosophy, if we defined categories by the features of a concrete object. The difficulties with the intermediate forms are connected with the definition of species and the extent of its variability. Nobody can deny their existence, but it is not clear what nomenclatorial status they will be given and how they will be used. Their taxonomic position is still a matter of opinion of different authors and will remain a matter of opinion until the question is resolved by accepted nomenclatorial procedures.

With the growing number of tests for identification purposes and the increasing amount of information accumulating, there arises the question of how to record it all. The development has proceeded from primary diagnostic cards (e.g., see Society of American Bacteriologists, 1957), to punched cards, from which it is only a step to electronic computers. The adoption of the principle that the value of individual characters is the same, leads to the utilization of statistical methods for the comparison of individual strains or taxons, and to the numerical expression of their similarity (Sneath, 1957b). If the correlations between strains or taxons are expressed numerically, then it is possible to express these relations graphically (Sneath, 1957b; Sneath and Cowan, 1958; Rhodes, 1961; and others) or also three-dimensionally in the form of taxonomic models (Lysenko and Sneath, 1959; Lysenko, 1961). These new methods not only offer a possibility of mechanized classification capable of handling a practically unlimited number and amount of material, but also make it possible for us to imagine more concretely the taxonomic relationships between groups of bacteria. The confusion in

theoretical questions is reflected in practical application of taxonomy, i.e., in the determination of bacteria. Wilson and Miles (1946) divided bacteriologists into "lumpers" and "splitters." There is nothing so easy to do as to describe new species. To give a reason for this description is, however, much more difficult.

For this reason I consider it useful to summarize some principles for determining or describing new bacterial species: (1) The determination should be based on the latest as well as earlier descriptions, and, if possible, compared with the initial original description. (2) Do not proceed according to a key, but compare with descriptions; base the determination on properties used for the group to which the microorganism belongs (bacilli by the taxonomy of bacilli, etc). (3) If the microorganism differs in some of its properties with that of a pertinent description, it is not necessary to describe a new species. (4) In describing a new species it is necessary to mention the properties in which the microorganism differs from those mentioned in the description; references and methods used should be cited. (5) If new tests are used, the newly found properties should be mentioned together with a description of methods used; the more characters given, the better. (6) New species should be described on the basis of the study of a number of cultures (i.e., isolations) from various materials. (7) If the bacterium is an intermediate form, a comparative identification should be given. (8) The description should be based on a comparison with related species, and, if possible, on a comparison with type cultures. The same holds true for the determination of varieties, etc.; it is not possible to make changes (e.g., in the rank of one species) that disregard the taxonomic construction of the entire genus. (9) If it is a new species, its description should be published in a leading microbiological or other appropriate journal. The type cultures should be selected and deposited in a recognized type-culture collection. (10) In all matters of nomenclature the International Code of Nomenclature of Bacteria and Viruses should be followed.

Although many of these principles are not entirely new (see Society of American Bacteriologists, 1957; Cowan, 1956a; Lysenko, 1959a; Buchanan *et al.*, 1958) many taxonomic papers are not in agreement with them. Some workers suppose that taxonomy has free entrance and that anybody, regardless of training or experience, may take part in it, but this is true only as long as the investigators follow the International Code and know the general and special problems. Even if many of these points still remain a matter of opinion, yet every opinion must be given serious consideration. Nobody would dare to characterize an enzyme without a knowledge of biochemistry, but many people without a knowledge of taxonomy want to introduce changes in the systematics of bacteria. Tax-

onomy is much more than the mere naming or identifying of cultures according to some key.

### **B. The Present Approach to the Taxonomy of Some Entomogenous Bacteria**

It is impossible in a limited space to enumerate all papers, changes, and problems concerned with the taxonomy of entomogenous bacteria. Some of the more recent papers are mentioned in the reviews by Steinhilber (1957), Tanada (1959), Krieg (1961a), and others. Therefore, I think it more effective to show how (in some cases from the standpoint of an insect pathologist) it is possible to apply the principles and theory I have been discussing.

The first case concerns the taxonomy of crystalliferous bacilli. As this group of bacteria are discussed in Chapter 2 of this volume and since there are some reviews of it (Heimpel and Angus, 1960b; Krieg, 1961b), I shall point only to basic taxonomic problems. In principle, there are two opinions as to the division of crystalliferous bacteria. Heimpel and Angus (1958, 1960a) separated these bacilli from the closely related *Bacillus cereus* and divided them into *B. thuringiensis* and its varieties (*thuringiensis*, *sotto*, and *alesti*), *B. entomocidus* Heimpel and Angus and its varieties (*entomocidus*, *subtoxicus*), and *B. finitimus* Heimpel and Angus. Toumanoff and Le Corroller (1959) recognized only *B. cereus* as the basic species and divided it into crystalliferous and acrySTALLIFEROUS groups with respective varieties. What is the difference between these two concepts? Practically it is only in nomenclature, because both systems are based on one property—the formation of crystals at the time of sporulation. All other divisions are according to the pathogenicity for different hosts, according to the formation of acetoin, lecithinase, and pigment, and to certain other properties. The formation of crystals cannot be regarded as a basic distinguishing character or as an “important” or “constant” character. Originally this property was considered a constant one. Later it was proved not to be, and now it is said that “most investigators agree that under normal conditions . . . the production of *crystals by pure cultures of crystalliferous bacteria* is a surprisingly constant character” (Heimpel and Angus, 1960b) [italics by O. L.]. In addition, we know that parasporal bodies are produced also by other species of bacilli. From the standpoint of taxonomy of the whole genus *Bacillus* Cohn, Toumanoff’s division is nearer the truth since it indirectly shows a certain phylogenetic relationship between *B. thuringiensis* and *B. cereus*. The concept by Heimpel and Angus is based on Bergey’s Manual (Breed *et al.*, 1957), where *B. anthracis*, for purely practical reasons, is tolerated as an independent species, although in the original taxonomy of Smith *et al.*

(1946) it is regarded as a variety of *B. cereus*. Now let us see how it is possible to show the taxonomic relationship of these bacteria by means of statistical methods.

On the basis of properties given in the literature, including pathogenicity and morphological characteristics, we obtain, by means of statistical methods (Sneath, 1957a, b), the percentage of similarity (Fig. 1). By the expression of these numbers in the form of a taxonomic tree (Fig. 2) we obtain a picture of these relationships. It is necessary to point out

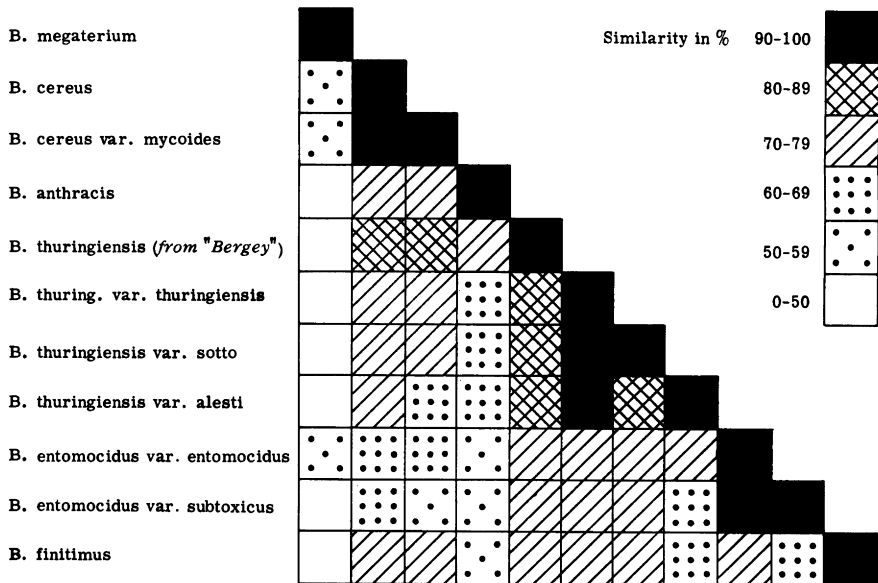


FIG. 1. Diagrammatic representation of the similarities (in per cent) of the species in the "*Bacillus megaterium*—*Bacillus cereus* Group." The Similarity values were calculated according to the species characteristics given in the descriptions presented by Bergey's Manual (Breed *et al.*, 1957), Heimpel and Angus (1958, 1960a), and Krieg (1961b).

that these calculations are not entirely accurate because they are based only on descriptions given in the literature, and thus not only have become generalized, but are mostly based on properties of only a single strain. Therefore we cannot, by using them, determine the variation range of the described species. The schemes that are presented here illustrate the suggested systems, not the actual variability of strains.

The tables show an obvious difference between *Bacillus megaterium* de Bary and *B. cereus* (50 to 60 percent), while differences between *B. cereus* and *B. thuringiensis*, *B. entomocidus*, and *B. finitimus* are much less (70 to 80 percent of similarity). From the standpoint of the taxonomy

of the whole genus, *B. megaterium* and *B. cereus* are closely related species, in comparison with the difference between, for example, *B. subtilis* Cohn and *B. circulans* Jordan. The following conclusions may be drawn:

From the standpoint of taxonomy of the whole genus it is possible to divide *B. megaterium* and *B. cereus* at the level of species. If we differentiate *B. anthracis* as an independent species from a purely practical point of view, quite logically we can similarly differentiate *B. thuringiensis*. Further distinguishing *B. entomocidus* and *B. finitimus* is not recommended, especially since their differentiation is based only on their

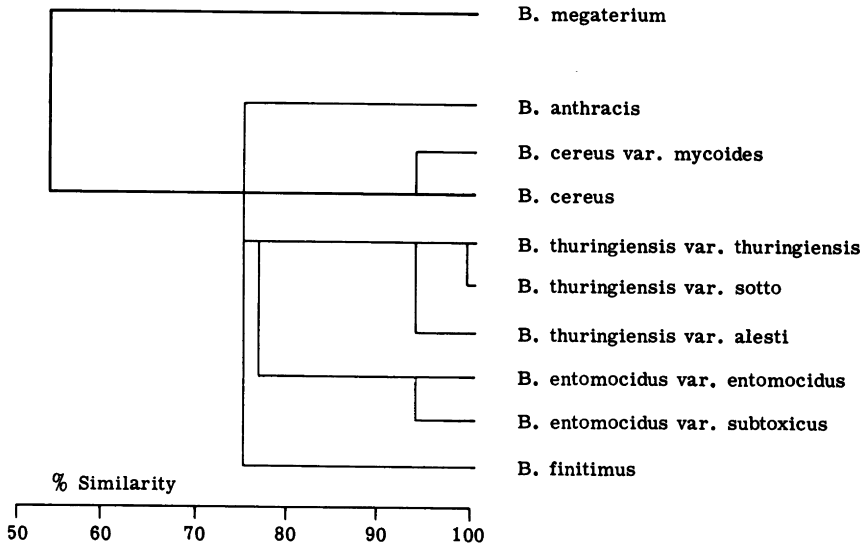


FIG. 2. A taxonomic tree showing the main relationships between the species given in Fig. 1.

somewhat inconstant properties, such as the production of lecithinase and acetoin. These properties in *B. thuringiensis* are not quite constant either (Lysenko, unpublished results). The division into varieties must be carried out very carefully and must be based on a greater number of strains than have been examined, because it is necessary to establish whether or not a variety is true and stable, i.e., whether it consists of strains that occur frequently or is only some intermediate or atypical culture. For example, the production of a red pigment is characteristic not only of "*B. alesti*," "*B. anduzae*," and "*B. euxoae*," but this pigment is also produced by some strains of *B. cereus* (Lysenko, unpublished results). It is analogous to the production of acetoin which, especially in bacilli, depends not only on the medium and elapsed time, but also on

the methods of keeping cultures. Already here we can see that there are a great number of changing values, and these may affect the pathogenicity for different hosts.

It is possible to divide bacteria into varieties and even lower taxa, but I am of the opinion that eventually we shall have to combine them again, because such divisions would not be easy to discern. We can still remember the situation that developed with the serological typing of *Salmonellae*. So if we want to divide crystalliferous bacilli in this way, we should not mix this activity with taxonomy. Each particular interest must be subject to the interests of the whole of taxonomy. Consider, for example, what would have resulted if we had divided species of bacilli according to their production of antibiotics.

As a second example, I shall mention the determination of entomogenous bacteria of the family Enterobacteriaceae. In this case also we find papers of different value. Unclear and, from the taxonomic standpoint, superfluous are the descriptions like those for *Escherichia klebsiellaeformis* Pesson *et al.* or *Parcobaetrum rhyncoli* Pesson *et al.* (see Krieg, 1961a). The first is invalid because it is a trinomial (Buchanan *et al.*, 1958, Rule 12a); both bacteria are obviously intermediate strains and do not correspond to the state of taxonomy of enterobacteria when presented in 1955. We could cite a number of similar cases (see Vago, 1959). On the other hand differences may be purely in the nomenclature. Steinhaus (1949, 1951) pointed out the coliform nature of *Coccobacillus acridiorum* d'Herelle. Lysenko (1958b) determined it as *Cloaca cloacae* var. *acridiorum* (d'Herelle) Lysenko, and Bucher (1959) classifies this microorganism as *Cloaca* type A. Both determinations are correct, as a matter of fact, because they well define the microorganism and it is always possible to tell the bacterium by its name and description. From the standpoint of the Bacteriological Code the first one is more correct; the second, however, corresponds to the group taxonomy used for enterobacteria (Report of the Enterobacteriaceae Subcommittee, 1958). Lysenko's division into a variety is superfluous, but it was done only to retain the historical name "*acridiorum*."

## V. THE TAXONOMY OF ENTOMOGENOUS BACTERIA IN THE FUTURE

It can be said that the taxonomy of entomogenous bacteria has made some achievements in the past ten years and so has participated in the construction of taxonomy in general. We have come so far that in many cases it is possible to redetermine and classify some old bacteria such as "*Coccobacillus acridiorum*" (Lysenko, 1958a), "*Streptococcus bombycis*" (Lysenko, 1958a), "*Streptococcus pluton*," and "*Bacterium eurydice*"

(Bailey, 1957), "*Pseudomonas septica*" (Lysenko, 1961), and others. This is a valuable contribution for it eliminates some of the names with the epithet "incertae sedis." On the other hand, we must take care that the newly formed systems do not become infected with thoughtless descriptions of species and complicated with excessive divisions. Many unclear descriptions still remain. I am afraid that, as pointed out by Steinhaus (1946a) in the case of certain bacilli, we shall never be successful in classifying some of the species described in the early literature of insect pathology.

The amount of work and the requirements to be expected in pursuing the taxonomy of entomogenous bacteria will naturally increase. The taxonomy of crystalliferous bacteria and that of *Bacillus popilliae* remain to be clarified. We do not know much about the taxonomy of symbiotic (mutualistic) bacteria in insects or of other microorganisms especially adapted to insects. We know relatively little of entomogenous anaerobes, of the ecology of bacteria in the insect gut, etc. Probably we shall have to revise our methods for the isolation of bacteria from insects, because it is likely that the methods used will not prove convenient or effective for all bacteria. It will be necessary to use more tissue-culture techniques. For recording results it will be more effective to use punch cards, and the use of statistical methods will help us to find better correlations between properties and taxons. In the future, computers will be used to a greater extent. Also the genetic interrelationships will play a greater role in our schemes of classification. However effective our methods become, and however specialized the bacteria we shall study, we must always keep in mind that bacteria, regardless of their source, are first of all bacteria, constituting a biological entity in themselves, and therefore we must have only one taxonomy—a taxonomy that must be simple and usable for everyone and that must have a stabilized inner order.

While the manuscript of this chapter was in press, there appeared several new papers and books dealing with the subject concerned. The author feels it would be very useful to mention here some of the most important of these. De Barjac and Bonnefoi (1962) published a paper in which a new scheme for the taxonomy of *Bacillus thuringiensis* was proposed; this paper has been discussed from the taxonomic point of view by Lysenko (1962). The 12th symposium of the Society of General Microbiology was dedicated to the general problems of bacterial taxonomy (Ainsworth and Sneath, 1962); most of the problems discussed in this chapter were also dealt with at the symposium. Cowan and Steel (1961) published very good identification tables, which, although they were constructed primarily for the identification of bacteria of medical importance, might be very useful for insect pathologists.

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# *Diseases Caused by Certain Sporeforming Bacteria*

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## I. INTRODUCTION

Insect pathologists have long been preoccupied with the possibility of utilizing microorganisms to control the abundance of those insect species whose activities bring them into competition with man. It is understandable then that interest should have been centered on forms able to persist in a dormant or quiescent stage outside the intended host insect. In the bacteria, a number of mechanisms aiding persistence have evolved; one of the most successful of these is the ability to form an endospore, which has been described as “a veritable fortress against most of the detrimental effects of the environment” (Oginsky and Umbreit, 1954).

The sporeforming bacteria have been subjected to much study, and

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