BIOLOGY OF PLANT LITTER DECOMPOSITION DECOMPOSITION DECOMPOSITION

Volume 2

edited by
C. H. Dickinson and G. J. F. Pugh

Academic Press
London and New York
A Subsidiary of
Harcourt Brace Jovanovich, Publishers

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1974
ACADEMIC PRESS . London and New York
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road,
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Card Number: 73-9457
ISBN: 0–12–215002–3

Text set in 11/12 pt. Monotype Imprint, printed by letterpress,
and bound in Great Britain at The Pitman Press, Bath

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Preface

In the past much emphasis has been placed on finding out what organisms occur in particular processes such as litter decomposition and in habitats such as soil. Within some disciplines the emphasis is moving towards autecological studies. Recent volumes have tended to be concerned with particular groups of organisms in specific habitats, such as fungi in soil, animals in soil and marine bacteria. We now see the need to synthesize the knowledge that has been obtained during studies of specific groups of organisms and to draw attention to their interrelationships in plant litter decomposition.

A central process in the life cycle of all green plants is the decomposition of their remains. During this decomposition many complementary and/or competing organisms are active. Frequently the processes of decomposition begin before the plant part senesces and the sequence of organisms involved is related to the type of plant material and the environment. Decomposition is then conditioned by the nature of the plant tissues, the range of organisms able to decompose these tissues, and the environment.

The arrangement of the chapters in this book follows the above pattern, and the quantity of material involved has made it necessary to divide the book into two volumes. In Part I, which constitutes Volume 1, the primary emphasis is placed on the type of litter. In this context litter is taken to include all plant remains, which range from still standing dead trees to the decomposing hyphae of fungi and cells of bacteria. Herbivore dung is considered in this section as digested litter.

The organisms involved in decomposition processes are discussed in Part II, which forms the first part of Volume 2. The treatment of each group is not meant to be exhaustive and may be vulnerable to criticism by specialists in the appropriate disciplines. However, our intention has been that the chapters indicate the range of structure and function of the organisms concerned. Biotrophic and necrotrophic parasites of higher plants, by their activities, are involved in the early stages of decomposition. It has therefore been found appropriate, when considering certain groups of organisms, at least to mention such parasites in the appropriate chapters.

The second part of Volume 2, Part III, is concerned with the environmental conditions under which breakdown occurs over the whole global surface. Terrestrial, freshwater and marine environments are considered separately. A further two aspects are anthropocentric: agriculture, with an emphasis on the importance of the saprophytic activity of plant pathogenic fungi; and the increasingly important composting of urban waste. We have allowed a small amount of overlap between certain chapters where we felt it desirable to give a balanced account within the particular treatment.

The editors wish to express their thanks to the chapter authors for their co-operation. Mrs S. J. Dickinson prepared the systematic index. Mrs M. Beck, Mr G. Maggs, Mrs V. Ross and Mrs B. Wallace have assisted in the preparation of this volume, and efficient secretarial assistance has been provided by Mrs J. Hall. We have also been greatly encouraged and assisted by Academic Press.

C. H. DICKINSON
G. J. F. PUGH

September, 1973

Contents of Volume 2

Contents of Volume 1

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Part II

The Organisms

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8

Bacteria

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I. Introduction

The gross function of a system can usually be expressed in simple general terms, and biological systems are no exception. The decomposition of plant litter can be described with a few equations which indicate that organic matter is ultimately broken down to carbon dioxide and water. However, this formalization does not tell us how the system operates and which control mechanisms keep the system in its dynamic state. Understanding a biological system requires knowledge about the organisms involved in the system, their characteristics and abilities, as well as their relationships and interactions. Taxonomy is the tool to produce much of this knowledge, and particularly to arrange the knowledge in a way which makes it available and which maximizes its content of relevant information. Any attempt to survey the bacteria involved in plant litter decomposition thus necessarily requires a taxonomic approach.

Taxonomy is based on three elements: classification, nomenclature, and identification. In this context the problems of identification are less significant as compared with classification and nomenclature, and the

discussion below is thus restricted to these two elements. Classification and nomenclature of bacteria operate with the *species* as the basic unit. True, this concept is by no means clear, as is obvious from the definition proposed by Buchanan (1957) which states that "a species of a bacterium is the type culture or specimen together with all other cultures or specimens regarded by an investigator as sufficiently like the type (or sufficiently closely related to it) to be grouped with it". This definition, like other attempts to define the bacterial species, gives rise to several controversial questions: Who is a competent investigator? What is meant by "like" or "closely related"? And how should "sufficient" be interpreted?

However, Buchanan's definition cannot be regarded as an inconsistent one. It expresses what every microbiologist knows or intuitively feels, namely that classifications of bacteria are never static and final, but dynamic and continuously changing. Moreover, Buchanan's definition can be applied to the different "technical" species that have been introduced into microbiology. Ravin (1963) defined three technically different species: (a) the *taxospecies*, which is a tight phenetic cluster produced when individual strains that have a high proportion of characters in common are grouped together; (b) the *genospecies*, which is a group of organisms which show mutual exchange of genes; and (c) the *nomenspecies*, which can be defined as a group of organisms that bear a certain binomial name. All these types of species have been used in the literature on soil bacteria, and hence also on litter-decomposing bacteria. The nomenspecies is distinctly connected with traditional taxonomy which applies a "downward" classification (dividing groups into subgroups and these into subgroups, etc.) into usually strictly monothetic groups. As characteristics which define groups one may find quite ordinary determinative features of the type "production of hydrogen sulphide" or "utilization of pentoses", etc. However, some extraordinary biochemical ability may also be used for group definition (e.g. *Pseudomonas methanica*) as well as specific ecological features as the habitat (e.g. *Enterobacter cloacae*). The taxospecies is due to application of numerical taxonomy which implies an "upward" classification (collection of individual strains into primary groups which are combined into larger groups etc.) into polythetic groups. Group definition in simple terms is difficult, and usually the circumscription of taxospecies is performed with a conventional classification into nomenspecies as reference. The same also concerns genospecies. Since exchange of genes is the basic criterion for the definition of a genospecies, reference strains are likely to be necessary. Again the reference system is usually provided by a conventional nomenspecies classification. In addition to exchange of genes, a narrow range of GC- (guanine and cytosine) content in the DNA is indicative of a genospecies. A better alternative criterion for a genospecies

is a high DNA homology. However, the necessary technique is more laborious than that for determination of the GC content. Information is thus usually limited to homology with a given reference organism. It follows then that application of DNA homology data involves merely a kind of verification of the nomenspecies by independent methods rather than the building up of a "pure" genospecies classification.

It is quite obvious that the three types of species described above often give rise to considerably different taxonomic practices when applied to certain groups of organisms. To take the Enterobacteriaceae as an example, the number of recognized nomenspecies is quite high. Utilization of the taxospecies approach already reduces the number of formal taxa, but since exchange of genes is known to take place between members of many taxospecies, a classification on the basis of genospecies would reduce the number of recognizable "species" still more. Alternatively,in groups of organisms which have been studied more superficially (i.e. the number of recorded characteristics is low), only a few still heterogeneous nomenspecies and/or taxospecies have been described. Most probably such "under-classified" taxa may contain several genospecies. There occur, of course, other cases where the three species concept corresponds quite well. For further discussion of this topic reference may be made to the paper of Jones and Sneath (1970).

As far as the taxonomy of litter-decomposing bacteria is concerned, the general approach seems still to follow traditional lines with the application of the nomenspecies as the basic unit. However, numerical methods have been employed quite frequently in recent years in soil microbiology (and the microbiology of decomposing organic matter) to verify earlier classifications and to increase distinction and usefulness of classifications. Similarly, genetic and chemical methods have been applied to characterize bacterial DNA for the same purposes.

Although a traditional classification into monothetic groups still provides the basic reference system for soil bacteria (and bacteria from corresponding environments), there is much complementary information available from application of modern techniques. This permits discussion of many groups of bacteria in quite distinct terms. For other groups, unfortunately, the picture is still unclear and sometimes even confusing, and hence, for these groups, the basis for a detailed discussion is non-existent.

II. Taxonomy

A. General

The bacteria which are involved to a significant extent in the decomposition of plant litter can be classified in the orders Pseudomonadales,

Eubacteriales, Actinomycetales, and Myxobacterales. Since the Actinomycetales will be treated in a separate chapter of this book (Goodfellow and Cross, Chapter 9), the following survey is limited to the other orders. This does not imply that these orders are discussed in full detail, the order structure has been chosen only for the sake of obtaining a logical disposition of the material. The species, as already indicated in the introduction, constitutes the basic unit in the taxonomy of bacteria, and main emphasis will therefore be given to the species level, and whenever necessary, to the generic level. Taxa of higher hierarchical ranks will be largely ignored.

B. Pseudomonadales

From the point of view of decomposition processes the most significant group in the Pseudomonadales is the genus *Pseudomonas*, which can be defined as consisting of Gram-negative, polarly flagellated rods with an oxidative sugar metabolism. Some *Pseudomonas* types are able to use denitrification as an alternative anaerobic mechanism for respiration. Organisms with a fermentative carbohydrate metabolism are definitively excluded from the genus. In addition, most pseudomonads are oxidase positive (oxidase test by Kovacs, 1956). Exceptions are *P. maltophila* (Stanier *et al.*, 1966) and certain plant pathogenic pseudomonads (Lelliott *et al.*, 1966).

A positive arginine dihydrolase reaction (Thornley, 1960), production of protocatechuic acid from quinic acid (Stewart, 1965), and production of fluorescent diffusible pigments are all characters which when they occur in combination with the primary criteria for *Pseudomonas* are strongly indicative for the classification of an organism as a member of this genus. However, these characters are not ultimate criteria. The genus *Pseudomonas* comprises non-pigmented as well as fluorescent organisms, and some pseudomonads lack a constitutive arginine dihydrolase system (cf. Stanier *et al.*, 1966). The degradation of quinic acid to protocatechuic acid is also a common, but not a universal feature, among the organisms classified as *Pseudomonas*.

The definition of *Pseudomonas* is thus relatively broad, and includes organisms which are mutually quite different. This is also indicated by the average range of the DNA base composition (De Ley, 1964). The range for *Pseudomonas* extends from 60% to almost 70% GC, which is a wide range as compared for instance with *Rhizobium* (60–62% GC). The fact that *Pseudomonas* seems to constitute a heterogeneous group has introduced the question as to whether it is justifiable to exclude some organisms from the genus. Davis and Park (1962) suggested the exclusion of *Comamonas*, i.e. organisms which otherwise fit the basic definition of *Pseudomonas*, but are

completely inactive against sugars. *Xanthomonas* has been separated on similar grounds (plant pathogenity, carotenoid pigments; Dowson, 1939). However, the separation of different groups from *Pseudomonas* only on the basis of one or two functional characteristics does not contribute to the homogeneity of *Pseudomonas*. The situation is similar for *Methanomonas* (ability to utilize methane as sole source of carbon), *Alginomonas* (capable of alginic acid degradation), *Cellvibrio* (capable of cellulose degradation), and *Hydrogenomonas* (ability to grow chemolithotrophically with hydrogen). Stanier *et al.* (1966; cf. also Hendrie *et al.*, 1968) have pointed out that all the organisms which have been classified into the genera mentioned above and further genera of the same kind could well be included in *Pseudomonas*, If a subclassification of *Pseudomonas* is felt advisable it should be based on other criteria, and it should, of course, create groups that are more homogeneous than is *Pseudomonas*.

In the seventh edition of Bergey's Manual (1957), the genus *Pseudomonas* comprised not less than 149 separate "species". Since then investigations using various named *Pseudomonas* cultures and/or fresh isolates of *Pseudomonas* from different environments, and statistical treatment of the results, have in general yielded only a few major clusters in which some of earlier named "species" have been combined (Rhodes, 1959; Lysenko, 1961; Colwell and Liston, 1961; Gyllenberg, 1965; Stanier *et al.*, 1966; Gyllenberg and Eklund, 1967).

Stanier *et al.* (1966), who have performed the most comprehensive study, accept four major groups of pseudomonads, namely the fluorescent group, the pseudomallei group, the acidovorans group, and the alcaligenes group. In addition to these groups only four further species are defined (*P. multivorans*, *P. stutzeri*, *P. maltophilia* and *P. lemoignei*). The fluorescent group constitutes one of the most extensively studied groups of bacteria. It includes the pathogens, *P. aeruginosa*, *P. fluorescens* and *P. putida*; for the last two species a number of biotypes have been defined. The fluorescent group is characterized by the ability to produce water-soluble green fluorescent pigments (pyoverdin), and to use different organic compounds as carbon and energy sources. Amongst other groups a higher potential capacity for breakdown of organic compounds is shown only by *P. multivorans* which seems, however, to be either geographically restricted or unable to compete successfully with fluorescent pseudomonads under ordinary soil conditions.

As already mentioned, a number of minor groups of bacteria have been separated from *Pseudomonas* on different grounds. Two of the groups, *Cellvibrio* and *Alginomonas*, are certainly involved in the degradation of complex organic molecules. Others, like *Methanomonas*, utilize and hence also decompose lower hydrocarbons. As a whole, these organisms

(*Pseudomonas* included) are characterized by relatively rapid growth, and versatility both as regards available nutrients and environmental factors.

The genera *Aeromonas* and *Vibrio* consist of polarly flagellated, oxidase-positive but fermentative organisms. An identification key for these organisms is presented by Bain and Shewan (1968).

C. Eubacteriales

1. Sporeformers

The members of the genus *Bacillus* can be defined as rod-shaped-organisms which produce endospores. They are usually Gram-positive, produce catalase, and are capable of sporulation under aerobic conditions (Wolf and Barker, 1968).

Non-sporulating variants have been described in several of the species. Biochemical reactions may in such cases need a supplementary study of the cell antigens (cf. Baillie, 1967; Norris and Wolf, 1961). The non-sporulating bacilli seem to constitute a link to *Lactobacillus* with *L. plantarum* as an obvious counterpart (Davis, 1964).

The present classification of the genus *Bacillus* is mainly based on the extensive report of Smith, Gordon and Clark (1952). Since then repeated approaches to the classification of bacilli have in general verified the conclusions arrived at by Smith *et al.*, and proposed changes are not essential. However, a new edition of their monograph (Gordon *et al.*, 1973) is due out shortly and it will widely be appreciated. In fact only minor modifications have been suggested to the characters applied for the definition of species, and the introduction of new taxonomic methods supplies additional detailed information.

Three subgroups can be recognized in the genus *Bacillus*; they are distinguished on the basis of the form of spores, the size of spores in relation to the vegetative cells, the thickness of the spore wall and the nutritional requirements of the vegetative culture. In the following description of the subgroups the modifications proposed by Wolf and Barker (1968) are noted. The first group (oval or cylindrical spores; sporangia not swollen; thin spore wall) comprises *B. subtilis* and variants, *B. cereus* and variants, *B. megaterium*, *B. licheniformis*, *B. coagulans*, *B. firmus* and *B. lentus*. The second group (oval or cylindrical spores; sporangia swollen; thick spore wall) includes *B. stearothermophilus*; some strains of *B. coagulans*, *B. laterosporus*, *B. polymyxa*, *B. macerans*, *B. larvae*, *B. popillae*, *B. lentimorbus*, *B. brevis*, *B. pulvifaciens*, *B. pantothenticus*, *B. alvei* and the *B. circulans* complex. *B. pantothenticus* cultures form approximately equal numbers of ellipsoidal and round spores. They are therefore intermediate between the groups 2 and 3. The third group (spherical spores; swollen sporangia;

complex nutritional requirements) is composed, besides the intermediate *B. pantothenticus* of only two species, namely *B. sphaericus* and *B. pasteurii*.

The major taxonomic problem relating to the genus *Bacillus* concerns the unclear relations of *B. circulans* to several other organisms mentioned above as members of the second subgroup of *Bacillus*. Intermediate strains, especially between *B. circulans*, on one hand, and *B. alvei*, *B. brevis* and *B. laterosporus*, on the other, are common. It is worth emphasizing that the relationship between *B. circulans* and *B. macerans* is also very close. All this may point to high variability and even genetic instability in the second subgroup of *Bacillus*, which can be considered as an indication of a state of active development (cf. *Lactobacillus plantarum*; Davis, 1964). This is of particular interest since active decomposers are found in this group as well as organisms which grow under extreme conditions of temperature.

Records of the thermophilic bacilli in the literature go back to the last century, and several types and "species" have been described (cf. Allen, 1953). In the monograph of Smith *et al.* (1952), the number of separate thermophilic species was, however, reduced to only two, viz. *B. stearothermophilus* and *B. coagulans*. Darland and Brock have recently (1971) described a new thermophilic species—*B. acidocaldarius*—which differs from all known bacilli in its very high GC value (62%). Therefore, it seems questionable whether this organism can be included in the genus *Bacillus* at all. The problem of the temperature requirements of thermophilic bacteria under natural conditions have been much discussed. Most probably these organisms may show alternative nutritional requirements, etc., as a response to changing environmental conditions, which would explain their occurrence also in habitats where extremely high temperatures are improbable. In any case, the resistance of spores to external environmental effects is correlated to the temperature relations of the organisms. Hence, thermophilic bacilli produce highly thermoresistant spores, which may be an ecologically significant feature, particularly under natural conditions.

In 1966 Larkin and Stokes published a report on psychrophilic bacilli. In another paper (Larkin and Stokes, 1967) four psychrophilic species were described, namely *B. psychrosaccharolyticus*, *B. insolitus*, *B. globisporus* and *B. psychrophilus*. Except for *B. insolitus* which was described as related to *Sarcina ureae*, the other psychrophilic species seemed to be related either to the *B. circulans* complex as such or at least to the second subgroup of bacilli. This is also obvious from the later work of Laine (1970) in which the observations of Larkin and Stokes were largely confirmed. In addition, Laine was able to isolate a few gas-forming psychrophilic bacilli which thus bear a resemblance to *B. polymyxa*. A further psychrophilic type, quite frequent in nature, described by Laine, also seems to belong to the second

subgroup of bacilli. Laine's data (1970) indicate that psychrophilic bacilli occur frequently in different natural habitats, among them plant materials. The ability of these organisms to maintain their metabolic activity at low temperatures points to their contribution to natural decomposition processes. The resistance of the spores of psychrophiles is quite low, and dependent on the temperature where sporulation takes place. Laine (1970) reported about three times higher D-values for psychrophilic spores produced at 20°C as compared with spores produced at 2°C.

As an illustration of further problems in the taxonomy of bacilli, the status of certain varieties of *B. subtilis* and *B. cereus* can be mentioned. For traditional reasons, particularly due to their specific pathogenity, *B. anthracis* and *B. thuringiensis* could well be considered as separate species. From strictly taxonomic points of view these organisms fit the definition of a variety, and should be regarded as varieties. *Sarcina ureae*, mentioned above, is a sporeformer, and available information indicates that, in the opinion of some workers, it should be classified among the bacilli, but its relation to the different subgroups of bacilli is not clear. In this connection the results of Rogers *et al.* (1970), who describe coccoid mutants of *B. subtilis* and *B. laterosporus*, are of special interest. On the other hand, the present definition of the genus *Bacillus* and the meaning of the generic name denote a rod-shaped micro-organism.

As compared with the genus *Bacillus*, the taxonomic information available for *Clostridium* is much less detailed. This group of strictly anaerobic sporeformers is not very homogeneous, and the genus includes organisms which cover a wide ecological and physiological range. A common general feature is, however, the versatile ability to attack different organic compounds as well as the high rate of the breakdown processes when they take place under optimal conditions.

From the point of view of plant litter decomposition, the pectinolytic and the cellulolytic species deserve attention. In addition to *Clostridium felsinum* which is usually mentioned in connection with flax retting, several other pectinolytic species have been described (cf. Lanigan, 1959). The separation of these species from each other is mainly based on differences in spore morphology, pigmentation, and the volatile compounds they produce from glucose. The pectinolytic clostridia reveal fermentation patterns varying from the butyric type to the acetobutylicum type, as well as intermediates. *C. felsinum* represents the acetobutylicum type, whereas *C. flavum* brings about a butyric acid fermentation. *C. laniganii* constitutes an intermediate to the two main metabolic types.

The cellulolytic clostridia can be divided into mesophiles and thermophiles. The organisms are however, obviously closely related, and this is confirmed by the type of fermentation. Both mesophiles and thermophiles

decompose cellulose with vigorous production of hydrogen, carbon dioxide and organic acids. The borderline between the mesophiles and the thermophiles remains somewhat indistinct due to the fact that some mesophilic species may grow at temperatures around 50°C, whereas the temperature optima of the thermophilic species remain around 60°C or even lower. From the ecological point of view an obvious difference exists. The thermophilic species seem to occur frequently in soil and on decomposing plant material, whereas the main habitat of the mesophilic species is the intestinal tract of herbivorous animals. However, the intestinal bacteria may be disseminated in soil by manure, etc. The most frequently encountered mesophilic species are *C. cellulosolvens*, *C. cellobioparum*, *C. omelianskii*, and *C. dissolvens*, whereas *C. thermocellum* and *C. thermocellulaseum* are the main thermophilic species. Differentiation of species is based on the ability to ferment glucose, motility, pigmentation and spore morphology.

2. *Enterobacteriaceae*

The Enterobacteriaceae are of interest in connection with plant litter decay (a) because organisms related to "*Aerobacter*" *aerogenes* occur frequently in nature and some seem to be active decomposers of complex organic compounds under natural conditions, and (b) because there are, in the genus *Erwinia*, organisms which colonize and decompose plant materials. Moreover the taxonomic problems relating to the correct classification and nomenclature of these organisms are quite intricate.

A comprehensive review of the genus *Erwinia* has been recently published by Starr and Chatterjee (1972). The obvious conclusions which can be drawn from this paper, and the background literature, are that the organisms involved can be divided into three main groups, namely (a) the amylovora group, (b) the carotovora group and (c) the herbicola group. Of these organisms the herbicola group is interesting because these organisms frequently colonize seeds, leaves and other living plant materials, and they thus constitute an "indigenous" population available to break down litter immediately it is formed. The organisms in the carotovora group are pectinolytic, and it has been suggested that these bacteria could be collected in the genus *Pectobacterium* (Waldee, 1945). In addition to the three main groups, some erwinias remain unclassified. These may be classified in further "species" or collected into a common group. The taxonomic problem follows from the fact that the different groups of erwinias mentioned above show affinities to other genera and tribes within the Enterobacteriaceae. Obviously the separation of the carotovora group into a new genus (*Pectobacterium*) solely on the basis of the pectolytic activity does not seem well motivated. Another alternative is to transfer the herbicola group to the genus *Enterobacter* (under the name *Enterobacter*

agglomerans, as suggested by Ewing and Fife (1972). However, the carotovora group could then also be included in *Enterobacter* on similar grounds, which would limit *Erwinia* to concern only the amylovora group. Starr and Chatterjee (1972) conclude that the erwinias are enterobacteria without any doubt, but that the question of retaining the genus as it now stands or of distributing its members among other genera of Enterobacteriaceae must await acquisition of additional information. Gardner and Kado (1972) have recently compared base sequence homologies of the DNA of *Erwinia* species and other members of Enterobacteriaceae. Their results do not support some of the recently proposed taxonomic divisions within the genus *Erwinia*, particularly certain combinations of species. Their DNA homology data together with GC values show that erwinias really form a loosely combined group of bacteria, often with no greater affinities to each other than to other enterobacteria. Gardner and Kado do not however support the including of the herbicola group in *Klebsiella* as proposed by Ewing and Fife (1972) since they found only 10% relative homology between *E. herbicola* and *Klebsiella aerogenes*. On the other hand, the homologies between *E. herbicola* and other erwinias were not much greater.

Aerobacter aerogenes was allocated to the genus *Klebsiella* in the classification scheme which Kauffmann published in 1959. This, however, led to misinterpretations since the earlier name was frequently applied to organisms which should be classified as *Klebsiella pneumoniae*. Kauffmann (1954) also introduced the two new genera *Cloaca* and *Hafnia*. A rearrangement was suggested by Hormaeche and Edwards (1960) who classified all these organisms in the genus *Enterobacter*, with *E. cloacae* as the type species, and *E. aerogenes* and *E. liquefaciens* as further members of the genus. As mentioned above, it has since been proposed that *Erwinia herbicola* should be grouped here and, consequently, renamed as *Enterobacter agglomerans* (Ewing and Fife, 1972).

The validity of Hormaeche's and Edwards' (1960) classification is, however, questionable in the light of a recent numerical analysis performed by Bascomb *et al.* (1971). These authors found that *E. aerogenes* showed strong affinity to *Klebsiella*, whereas *E. liquefaciens* was most closely related to *Serratia*. Again, more information is obviously needed before the taxonomic position of the organisms in question can be finally settled.

3. Other Eubacteria

The taxonomy of the genus *Agrobacterium* has been discussed by De Ley *et al.* (1966). These authors suggest that only three species can be recognized in the genus, viz. *A. radiobacter* (the crown-gall bacterium is considered as a variety *A. radiobacter* var. *tumefaciens*), *A. rhizogenes* and *A. pseudotsugae*. The last organism was represented by only one strain in the

study of De Ley *et al.* (1966), and its relation to the genus *Agrobacterium* could not be positively defined. *A. radiobacter* and the crown gall variety show, on the basis of the GC percentage, a very close relationship to *Rhizobium leguminosarum*, but *A. radiobacter* can be separated from the latter by its ability to produce 3-ketoglucosides. Rhizobia are reported to show negative 3-ketoglucoside reactions (*Bernaerts* and *De Ley*, 1963), but the fact that the same authors noted later that *R. leguminosarum* produces some 3-ketolactose from lactose actually leaves open the extent to which *A. radiobacter* and *R. leguminosarum* can be reliably distinguished.

The taxonomic position of *Achromobacter* is still confusing. The problem has been discussed in detail by Ingram and Shewan (1960). The difficulty is that in earlier literature achromogenic pseudomonads were frequently referred to as "achromobacteria". At the same time the definition of the genus was unclear since no type cultures of *A. liquefaciens*, which was assigned as the type species of *Achromobacter*, were available. However, later Tulecke *et al.* (1965) were able to isolate an organism which corresponded almost exactly to the original description of *A. liquefaciens*. Tulecke *et al.* suggested that the genus *Achromobacter* would be retained with the re-isolated, motile, peritrichously flagellated strain as type species. On the basis of a broad computer survey on *Acinetobacter* and related genera, Thornley (1967, 1968) is of the opinion that the name *Alcaligenes* should be retained for the non-motile, Gram-variable, coccoid bacteria usually referred to as the *Achromobacter-Alcaligenes*-group. The human and animal pathogens also described as "achromobacteria", are quite different from this group. As shown by De Ley *et al.* (1967) these organisms form a distinct cluster which includes *Acinetobacter anitratum*. Another main group has been described by these authors. It includes the *Alcaligenes* group referred to above, and as a further subgroup certain strains of marine origin. The taxonomic position of "*Achromobacter*" seems still to be confusing.

There has been much disagreement concerning the correct taxonomic position of the genera *Corynebacterium* and *Mycobacterium*. Waksman and Henrici (1943) made a compromise and excluded *Corynebacterium* from the Actinomycetales, but retained *Mycobacterium* among the Actinomycetes. Jensen (1952) defined two "levels" of corynebacteria, viz. *sensu stricto* and *sensu lato*, the former including mainly the diphtheroids of animal origin whereas the latter comprises the diphtheroids, the plant pathogenic corynebacteria, and the saprophytic group. For Jensen's saprophytic group of corynebacteria Conn and Dimmick (1947) proposed the generic name *Arthrobacter* with *A. globiformis* as the type organism. However, the organisms related to *A. globiformis* are characterized by weak (if any) ability to attack carbohydrates. Clark (1952) thus suggested that saprophytes

which utilize carbohydrates vigorously, and some of which are cellulolytic, should be included in the genus *Cellulomonas*. Cummins and Harris (1958) noted that the animal strains of corynebacteria were characterized by a distinctive pattern of sugar and amino acid components in their cell walls, very similar to the pattern of cell wall components found in strains of *Mycobacterium* and *Nocardia*. In a later paper, Cummins and Harris (1959) analysed the cell walls of seven *Arthrobacter* species. Their conclusion was that there cannot be any close relationship between these organisms and the corynebacteria proper. The results also indicated heterogeneity in the genus *Arthrobacter*. Five of the species studied fell in the same group as *A. globiformis*, whereas *A. simplex* and *A. tumescens* differed from other species and grouped together.

Jensen (1952) put forward the hypothesis that the arthrobacteria form a primitive and versatile group from which other groups of corynebacteria have developed by loss of characters. They may also be ancestors of the Actinomycetes, with some of which they share certain features. Recent investigations have shown, however, that the arthrobacteria are not very closely related to diphtheroids of animal origin, and have confirmed that the borderline between the arthrobacteria and certain groups of Actinomycetales is far from being distinct and easily definable (Gordon, 1966; Bousfield, 1972).

Application of numerical taxonomy (Nigel da Silva and Holt, 1965; Goodfellow, 1967) has shown that phenetic similarities between members of *Arthrobacter* may be fairly low (e.g. between *A. tumescens* and *A. globiformis*). Zagallo and Wang (1962) found that *A. globiformis* and *A. ureafaciens* rely mainly on the operation of the Embden-Meyerhof-Parnas pathway, whereas *A. simplex* requires intermediate formation of gluconate.

Skyring and Quadling (1970) drew attention to the fact that the arthrobacteria can be divided into two groups on the basis of nutritional requirements. *A. terregens*, *A. flavescens* and *A. duedecades* are nutritionally exacting, and thus separable from the nutritionally non-exacting species. Further work (Skyring and Quadling, 1969) has indicated certain difficulties in the practical differentiation between *Agrobacterium*, *Rhizobium* and *Arthrobacter*, which also show, however, similarities in the GC content of their DNA. Principal component analysis of representative material of cultures from these three genera has shown (Skyring *et al.* 1971) that separation of the genera can be based on the abilities to use various carbohydrates and amino acids or to grow under various conditions.

As regard other eubacteria, it may be mentioned that taxonomic surveys of micrococci have recently been made by Baird-Parker (1963) and Rosypal *et al.* (1966). The taxonomic position of *Flavobacterium* is very problematic. The genus includes two main groups of organisms: (a) organisms with GC

values of 30–40% and (b) organisms with GC values >50%. The first group seems to represent atypical cytophagas, and the other group may also partly be allocated in some other genus. McMeekin *et al.* (1972) have suggested a simple scheme for the tentative classification of yellow pigmented rods in "low GC types" versus "high GC types". Bousfield (1972) has shown that some flavobacteria of the "high GC type" (% GC 65–67) obviously are coryneforms. These organisms could either be placed in *Arthrobacter* or in a new genus which would replace the rearranged genus *Brevibacterium*. The type species of the last mentioned genus seems to be better placed in *Arthrobacter*, and then the name *Brevibacterium* would be illegitimate.

The taxonomic position of the "low GC" flavobacteria is discussed under the heading Myxobacterales. Some of these bacteria seem actually to be atypical cytophagas (cf. e.g. Mitchell *et al.*, 1969).

D. Myxobacterales

The myxobacteria are usually described very superficially in textbooks and monographs with some general references to their strange morphology and their supposed importance in decomposition processes. During the last decade there has been an obvious increase in the interest devoted to these organisms which is reflected in many suggestions for taxonomic rearrangement.

In Bergey's Manual, 7th edition (1957) the order is divided into five families on the basis of occurrence and characteristics of resting cells, cysts, and fruiting bodies. A successive change to higher complexity of these bodies is obvious. *Cytophaga* occurs only in the vegetative state, *Sporocytophaga* forms resting cells, but they are not condensed into fruiting bodies. In Myxococcaceae and Archangiaceae the resting cells are aggregated into special fruiting bodies, and, finally, in Sorangiaceae and in Polyangiaceae the primary resting cells are contained in secondary cysts which are attached to fruiting bodies. This logical spectrum of increasing morphological complexity was not, however, followed in the classification key of Myxobacterales in the last edition of Bergey's Manual. For some less obvious reason *Sporocytophaga* was included in the family Myxococcaceae which was listed as the last family after the cyst-producing types:

Family I.	Cytophagaceae:	No resting cells or fruiting bodies.
Family II.	Archangiaceae:	Resting cells on fruiting bodies.
Family III.	Sorangiaceae:	Resting cells in cysts on fruiting bodies
Family IV.	Polyangiaceae:	Resting cells in cysts on fruiting bodies
Family V.	Myxococcaceae	(including *Sporoctyophaga:* free resting cells): resting cells on fruiting bodies.

The invalidity of this classification became immediately obvious when GC analyses were applied to the group. The GC contents divide the order into two quite distinct groups with *Cytophaga* and *Sporocytophaga* in one group (GC contents in the range of 35–42%) and the fruiting body forming organisms in the other group (GC contents in the range of 68–72%). The difference is so clear-cut that the relation between both the groups already looks somewhat dubious. However, for the sake of the historical background a discussion of the cytophagas is included under the present heading.

It has been confirmed that some of the bacteria, earlier classified as "flavobacteria", a vaguely defined group of organisms, actually belong to the *Cytophaga-Sporocytophaga* group (Hendrie *et al.*, 1968; Mitchell *et al.*, 1969; Weeks, 1969), and this group is obviously very closely related to several other types of "gliding bacteria" (Lewin and Lounsbery, 1969; Mandel and Lewin, 1969; Fager, 1969; and Colwell, 1969). Lewin (1969) suggested that all these organisms should be collected into one family, Cytophagaceae (or Flexibacteriaceae) including the genera: *Cytophaga*, *Flexibacter*, *Saprospira*, *Flexitrix*, *Herpetosiphon*, *Sporocytophaga*, "*Sphaerocytophaga*" (or *Fusobacterium*) and *Microscilla*. In view of the distribution of GC content within these genera, the proposed classification seems questionable. The data of Mandel and Lewin (1969) indicated following figures for the GC contents: *Cytophaga* 34–41%, *Flexibacter* 30–47%, *Microscilla* 32–45%, *Saprospira* 35–48%, and *Herpetosiphon* 45–53%. The numerical analysis of Colwell (1969) indicates possibilities for some rearrangement. Since high similarity values (>80%) were found for strains assigned to *Cytophaga*, *Flexibacter*, and *Microscilla*, particularly with strains in the range of 35–40% GC, several strains labelled *Flexibacter* or *Microscilla* may in fact belong to *Cytophaga*.

For the myxobacteria which produce fruiting bodies a taxonomic revision has been proposed by McCurdy (1970). The major point in McCurdy's scheme is a redefinition of the family Polyangiaceae to include organisms with cylindrical cells. As a consequence the organisms earlier classified as Sorangiaceae are transferred to Polyangiaceae, and a new family, Cystobacteraceae, is proposed for sporangial myxobacteria ("sporangial" here refers to the formation of cysts containing resting cells or microcysts). McCurdy's revised scheme follows the logic of the gradual morphological change from one group to next, and seems to constitute a more reliable approach to the taxonomy of the fruiting myxobacteria than the classification followed in the seventh edition of Bergey's Manual. McCurdy's (1970) scheme is presented here for comparison:

I. Vegetation cells tapered, microcysts (slime encapsulated myxospores) produced.
  A. Microcysts spherical or oval. Myxococcaceae.

B. Microcysts rod-shaped.
   1. Microcysts not in sporangia. Archangiaceae.
   2. Microcysts in sporangia. Cystobacteriaceae.

II. Vegetative cells of uniform diameter with blunt, rounded ends. Myxospores resemble vegetative cells. Polyangiaceae.

III. Ecological Aspects of Bacterial Litter Decomposition

As a result of varying conditions plant litter decomposition may show different characteristics in different ecosystems, but in most cases it is characterized by periodicity, the accumulation of a huge biomass of decomposer micro-organisms and the resynthesis of organic compounds which may be less degradable than the original matter. Since the microbial biomass is also broken down successively the decomposition process is perhaps most correctly described as a chain process which ultimately combines with the nutrient cycles and even the primary production processes.

The successions of micro-organisms in litter decomposition are primarily determined and controlled by the abilities of the micro-organisms present to utilize available organic compounds, but partly also by the response of the organisms to changes in the environment caused by the decomposition process. The subject is extensive (cf. Alexander, 1964, 1968) and the following brief survey is presented in order to indicate the role of the bacteria involved in these processes.

The first compounds which are utilized in dead organic litter are water soluble nitrogenous compounds, sugars, and various organic acids. However, these compounds are readily transported by rainwater into deeper litter layers. It seems likely, therefore, that these compounds are: (a) utilized *in situ* by the phylloplane population, the development of which on the surface of living leaves and needles is controlled by exuded sugars and easily available nitrogenous compounds; and (b) extracted from the surface litter and transported to deeper litter layers where they are consumed either by the organisms originating from the litter itself or by the indigenous bacterial population of soil.

The bacteria which inhabit the surface of leaves and other aerial plant organs are mainly chromogenics, and among them *Erwinia herbicola* seems to be particularly significant. As with the enterobacteria in general, *E. herbicola* is stimulated by sugars, and consequently by the exudates in the phylloplane of living plants, and comparable extracted compounds. Stout (1958, 1960, 1961), in a series of extensive investigations of the bacterial populations of natural grasslands, cultivated pastures, and various forest habitats, reported an abundant occurrence of "flavobacteria" with an

oxidative sugar metabolism in the phylloplane of grasses and in the upper litter layers in forests. This points to the particular importance of the phylloplane as a source of litter-colonizing organisms of the *Flavobacterium-Flexibacter-Cytophaga* complex. Actually the chromogenic bacteria can be followed into deeper litter layers, where they are mixed with the indigenous population, but may occur abundantly as decomposers of cellulose and other polymeric compounds.

The soil bacteria are usually divided into two functional groups: (a) the indigenous organisms, whose numbers in soil are supposed to remain unaffected by the litter, and (b) the zymogenous organisms, which are actively involved in litter decomposition and which, therefore, increase to very high numbers during periods of litter decomposition. The zymogenous organisms do not of course disappear completely from the soil when decomposition slows down, and probably the leaching of water-soluble compounds from recently fallen litter may cause an activation of the retained zymogenes, which take over the next step of the degradation. This concerns the more easily decomposable polymeric compounds, including the pectins, hemicelluloses, cellulose and chitin.

The abilities to degrade these polymeric compounds are widespread among fungi and Actinomycetes but among bacteria these activities are restricted to a few genera or only a few species in certain genera. In Pseudomonadales cellulolytic organisms are found in the genus *Cellvibrio*. However, as pointed out in the taxonomic survey, the validity of this genus is unclear, and the only conclusion thus is that cellulolytic pseudomonads occur. Among the coryneforms, *Cellulomonas* represents a homogeneous taxonomic group (Nigel da Silva and Holt, 1965). In Eubacteriales other cellulose-decomposers are found among the sporeformers. It seems, however, that the activity of these organisms is mainly restricted to extreme environmental conditions. The cellulolytic clostridia in soil are thermophiles. Mesophilic species may be introduced by the faeces of herbivorous animals, but this route of colonization is hardly very important except for extensively manured field soils.

Among the aerobic bacilli there are active decomposers of starch, pectins and xylans. Mishustin and Mirsoeva (1968) are of the opinion that in soils where biological processes are at equilibrium, most of the bacilli are present as spores but that sporeformers multiply during later stages of mineralization of plant litter. It is interesting to note also that bacilli may be connected with the breakdown and recirculation of biomass. *Bacillus circulans* for example, was found to produce 1-3-glucanase lytic against fungal and yeast cell walls (Horikoshi *et al.*, 1963; Tanaka and Phaff, 1965). In Myxobacterales, i.e. the complex of fruiting "slime bacteria", flexibacteria, and flavobacteria, the ability to decompose polymeric organic

compounds seems to be more evenly distributed among the various organisms. The ubiquitous distribution in living and dead plant material of the "*Flavobacterium-Flexibacter-Cytophaga*" group was noted above, and fruiting myxobacteria are widespread in soil, on bark and decaying material as well as on dung (Peterson, 1969). Among the *Cytophaga*-species for instance *C. hutchinsonii*, *C. aurantiaca*, *C. rubra*, and *C. tenuissima* are reported to be active cellulose decomposers. *Cytophaga johnsonii* is known to be a chitinoclastic organism. Other types of cytophagas show activity against various organic compounds and they may be highly proteolytic, pectinolytic and amylolytic. In addition to *Cytophaga* and *Sporocytophaga* several other fruiting myxobacteria are cellulose decomposers. Moreover, these organisms are known to be highly bacteriolytic, mycolytic and nematodolytic. However, there seems to be a certain specificity between the attacked organism and the lytic myxobacterium (Stanier, 1942, 1947; Dworkin, 1966; Webley and Jones, 1971).

Although bacteria which are capable of degrading various polymeric organic compounds are widespread in nature, the existing environmental conditions may limit bacterial activity. In most environments fungi obviously constitute the primary decomposer population of plant material, whereas bacteria appear as a secondary population. This particularly concerns forest soils, where the predominance of fungi is well documented. The predominance of fungi as decomposing agents in forest soils is partly due to high acidity, but also to the fact that tannic compounds inhibit bacterial enzymes (Basaraba and Starkey, 1966; Henis *et al.*, 1964).

Bacteria exert considerable activity in the breakdown of fungal mycelium and similar materials. Different techniques to elucidate microbial activities in soil (such as the examination of buried cellulose films), have indicated that bacteria are particularly abundant around fungal hyphae (Tribe, 1957). In addition to the myxobacteria other bacteria, especially certain Streptomycetes, are strongly mycolytic.

Most reports on bacteria in connection with litter decomposition are descriptive and information is given only of relative numbers of various bacteria in decaying organic matter. For a few groups of bacteria more exact information is available. First, it is obvious (e.g. Rovira and Sands, 1971) that fluorescent pseudomonads are distributed unevenly in soil, and that these bacteria are associated particularly with organic matter. In the presence of readily utilizable nutrients fluorescent pseudomonads usually show a very rapid increase in numbers under soil conditions, but as soon as maximum cell density is reached they disappear rapidly (Eklund, 1970; Eklund and Sinda, 1971). This may point to an efficient utilization of useful substrates, which obviously causes a change in the environment so that the organisms are suppressed. The bacilli are another group of bacteria