# GENETICS OF BACTERIAL DIVERSITY

Genetics of Bacterial Diversity

We apologise for a minor printing error on the cover of this book.

The correct authorship is DAVID A HOPWOOD and KEITH F CHATER, as given on the title page.

The error will be corrected in future printings,

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# GENETICS OF BACTERIAL DIVERSITY

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# *Preface*

*E. coli* K-12 is a marvellous subject for genetical research. Without the flowering of molecular genetics that followed Lederberg and Tatum's classical discovery of sex in this simple bacterium, it is difficult to imagine that we would know so much about the structure, organization and expression of genes as we do today. (Even the discovery of such marvels of eukaryote genetics as introns, homeoboxes, or oncogenes depended directly on the development of the K-12 strain and its accessory DNA elements for the cloning and analysis of recombinant DNA.) But *E. coli* K-12 does not do everything. It does not degrade complex phenolics, photosynthesize, fix nitrogen or produce antibiotics or light. It does not differentiate to form stalks, spores or fruiting bodies. Nor does the K-12 strain parasitize animals or plants. Other bacteria do, and the application of molecular genetics is leading to an understanding of these phenomena. Such is the subject matter of this book.

For some time, we had felt the time to be ripe for a collection of articles on the genetics of a range of interesting things that bacteria do. But we were daunted by the conviction that such a book would need to contain, as a background, a detailed description of the full range of the "mainstream" topics of bacterial genetics that *E. coli* represents, since there appeared to be no suitable up-to-date textbook in this field. This deficiency was recently remedied by the publication of the book, *Genetics of Bacteria*, edited by Scaife, Leach and Galizzi (Academic Press, 1985); and the comprehensive and specialized compilation, *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by Neidhardt *et al.* (American Society for Microbiology, 1987). Our book is thus able to concentrate on the rapidly developing field of "non-K-12" bacterial genetics that is largely outside the scope of these other texts.

After an introductory chapter outlining the phylogenetic relationships of bacteria and the range of metabolic, behavioural and developmental phenomena displayed by them, Chapter 2 reviews the genetic processes found in bacteria generally, and Chapter 3 discusses a range of genetic techniques that have been used to analyse the various special systems described in the body of the book. The book is not meant to be exhaustive. Not only is our choice of topics deliberately eclectic, but we have encouraged the authors of most of the chapters to deal with a particular example of each topic in detail, rather than reviewing everything that is known about the phenomenon, such as light

production or sporulation, in all bacteria that display it. In this way, we hope that the book will give a strong flavour of the biology of bacteria and the ways in which the techniques of genetics are used to study them, which will be of interest to a wide range of scientists, rather than providing an exhaustive review of any particular topic for the specialist. In this vein, we asked authors to choose, wherever possible, key references and reviews to cite in limited bibliographies, rather than charting every step in the development of the subject.

Chapters 4–20 can be read in any order, even though we have grouped them into four main subject areas. Thus, Chapters 4–9 deal with various special metabolic capabilities characteristic of certain groups of bacteria (light production, photosynthesis, nitrogen fixation, antibiotic production, degradation of aromatic compounds and mercury resistance); Chapters 10–12 describe the developmental processes of cell-cycle associated motility, sporulation, and specialized colonial behaviour; Chapters 13–16 cover four components of bacterial pathogenicity for animals; and Chapters 17–19 deal with pathogenic and symbiotic interactions of bacteria with higher plants. Chapter 20 stands by itself. It is written by a population geneticist and explains, for the rest of us, mainly molecular, geneticists, some of the concepts and the progress now being made in the application of population genetics to bacteria.

As Hodgson points out in Chapter 1, the bacteria studied by geneticists cover only a narrow part of the whole phylogenetic spectrum. The glaring omission in current bacterial genetics is the study of archaebacteria. These prokaryotes grow at extreme pH values, temperatures and salt concentrations and have important metabolic capabilities such as methanogenesis and chemolithotrophy. They are clearly far removed phylogenetically from other groups of bacteria, and show some novel genetic properties; for example, their ribosomes have features intermediate between those of eubacteria and eukaryotes. A start has been made on their genetics and, if there were to be a second edition of this book, it is to be hoped that they would figure prominently in it.

We have deliberately avoided any unnecessary justification for research on bacterial genetics arising from its biotechnological opportunities, real or conjectural, although work on a large proportion of the organisms covered in the book—including of course the pathogenic systems—has in fact been motivated by anthropocentric considerations, and several applications have been discussed in the appropriate chapters. They range from the construction of *Pseudomonas* strains, able to degrade novel hydrocarbons polluting the environment, to the use of *Agrobacterium* as a microbial route to the genetic engineering of plants.

We hope that the book will be of interest to microbiologists wishing to catch up on the genetic basis of some of the classical phenomena of bacteriology, and geneticists unfamiliar with some of the things that bacteria can accomplish.

We hope it will be useful to undergraduates, graduate students and research scientists at many levels. Above all, we hope that it will help to dispel the sometimes tacitly assumed opinion that modern genetics has left prokaryotes behind to concentrate on eukaryotes. Eukaryotes are amazing genetic systems, but so too are bacteria!

We should like to thank the authors of the various chapters for joining with us in producing this book, for preparing their manuscripts promptly and for responding in a friendly way to our editorial suggestions. We are especially indebted to Anne Williams for the considerable secretarial task involved at all stages from the original conception of the book to its completion, and Gina Fullerlove of Academic Press for her efficient and sympathetic handling of the whole project.

David Hopwood
Keith Chater

Norwich, April 1988

Section **I**

# *Introductory Chapters—the Diversity of Bacteria and of Bacterial Genetics*

Bacteria are typified by the very small size of their cells, which are usually of the order of 1 $\mu$m in diameter, and the relative simplicity of their structure—they do not contain mitochondria or chloroplasts, nor is their genetic material contained within a nuclear membrane. The two bacterial kingdoms, eubacteria and archaebacteria, differ radically in their cell wall organization, and substantially in the details of their transcription and translation systems. In these latter features, archaebacteria are more like eukaryotes than eubacteria. This, and their remarkable ability to survive in various extreme environments, are now attracting considerable research interest to the archaebacteria. As yet, however, systems for their genetic analysis are not well advanced, a technical obstacle shared with many of the lesser-known groups of eubacteria whose remarkable biochemical, structural and ecological attributes are described in Chapter 1. The phylogenetic relationships of bacterial groups displayed in Fig. 2 of Chapter 1 reveal that those few bacterial groups for which extensive genetic analysis is already possible all fall in one sector of the phylogeny containing the purple group of Gram-negative bacteria (which includes *Escherichia coli*) and the Gram-positive bacteria.

The availability of systems of genetic analysis has allowed characterization of many basic genetic features of these groups, such as genome size and

circularity; the strategies of plasmids, transposons and phages; the various means by which genetic exchange can take place; and the means by which gene expression occurs and is regulated. Chapter 2 shows how this has led to a more fully substantiated view of the generalities of bacterial genetics than could be obtained by the study only of the single *E. coli* paradigm.

In briefly summarizing the overall genetic phenomenology of these bacteria in Chapter 2, and in dealing with special attributes of particular bacteria in later Chapters, it is inevitable that details of the techniques by which the discoveries were made should be omitted. This deficiency is redressed in Chapter 3, which specifically describes the techniques employed in molecular genetic studies of various bacteria, and serves as a reference point and glossary for the technical background of the more specific chapters that make up the main body of the book. It is clear that, while the use of the wonderful *E. coli* cloning and analytical systems provides great benefits in the study of all organisms, the most striking advances are made when recombinant DNA technology and allied techniques are also well-developed in the organism under scrutiny. Fortunately, the persistence of aficianados of different bacteria has resulted in the extension of *E. coli*-derived technology into most purple Gram-negative bacteria via wide host-range plasmids and transposons, and its use in targeted modifications of the genotype of the relevant organism by gene replacement or disruption. It has proved less straightforward to introduce *E. coli*-based technology into Gram-positive bacteria, and there has been much more emphasis on the exploitation of the native genetic elements and genetic features of these organisms in developing molecular genetic techniques for them. As a result, efficient plasmid- and phage-based cloning systems and well-developed transposon mutagenesis for Gram-positive bacteria are available both for the low and the high (G + C) divisions of this group.

Chapter 1

# Bacterial Diversity: the Range of Interesting Things that Bacteria Do

**D. A. HODGSON**

## I. INTRODUCTION

The aim of this chapter is to provide a bacteriocentric overview of the role of bacteria in nature. Hence the biotechnological roles of bacteria; both ancient and modern, are excluded, as are "bacteria as model systems". Rather I hope to show that bacteria are so interesting in themselves that there is no need to justify their study by recourse to some principle of anthropocentric utility.

Inevitably, this chapter contains a personal, possibly idiosyncratic, selection of topics. The chapter is organized into four main sections. In Section II, I discuss the diversity of bacteria in terms of the multiplicity of ecological niches that they fill. This is followed by a short discussion of the recent triumph of bacterial phylogeneticists in the assembly of a molecular phylogeny of bacteria using analysis of 16S rRNA and 5S rRNA (Section III). This allows us in Section IV to discuss the evolution of homologous and analogous structures, physiologies and behaviours in bacteria. Finally, there is a discussion of phenomena that might not have been expected to be present in bacteria (Section V).

## II. ECOLOGICAL NICHES

Bacteria can be found almost everywhere. This remarkable ubiquity results from and reflects their ability to gain energy and the necessary building blocks of life from diverse sources, and the evolution of novel physiologies and behaviours to allow life in extreme environments.

### A. Bacterial Nutrition is Diverse

*1. Inorganic sources of carbon, nitrogen and energy can be exploited by some prokaryotes.* The ability of bacteria to gain energy and reducing power by the oxidation or reduction of simple minerals or inorganic salts (chemolithotrophy) has been well documented since Winogradsky's report of 1890, but its investigation has largely been limited to biochemistry and physiology. Only recently has the analytical power of genetics begun to be applied to the methane oxidizers, the sulphur oxidizers and the carbon dioxide reducers (the methanogens). This is because many of these bacteria are obligate chemolithotrophs, difficult to culture on solid media and very slow growing. The many advances in gene cloning have allowed the transfer of genetic material to more amenable hosts where molecular analysis can now take place. The discovery that the methanogens belong to a third primary kingdom, the archaebacteria, separate from both the eubacteria and the eukaryotes, may have repercussions for the genetic analysis of these organisms.

Many bacteria can fix elemental nitrogen, an ability not found in eukaryotes. The complexity of the intensively studied gene system that determines this ability (Chapter 6) illustrates the general principle that nutritional simplicity is allied to genetic complexity.

A more limited range of bacteria are able to obtain all their energy for $CO_2$ reduction from light (Chapter 5). Some other bacteria can use light as an auxiliary energy source: for example, members of the Halobacteriaceae—an archaebacterial group—use bacterial rhodopsin to generate ATP. Both these groups of light-exploiting bacteria exhibit phototaxis; the mechanisms of which have been the subject of a recent review (1).

*2. Some bacteria can utilize toxic compounds.* Most bacteria are heterotrophs—they depend on complex sources of carbon and nitrogen for nutrition and energy. Amongst them, many have characteristics unique to the prokaryotic world. Some can use organic compounds that are toxic to other organisms to generate reducing power and release energy: thus, the abilities of one group, the pseudomonads, to deal with xenobiotics are described in Chapter 8. Plants, fungi and bacteria produce a very diverse range of compounds. So it is not surprising that the bacterial world already contains organisms which can deal with unusual chemicals. For example, *Streptomyces achromogenes* var. *streptozoticus* produces streptozocin (Fig. 1). Within this compound, which contains a dinitrogen bond, there lurks the active moiety of *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine, a most powerful mutagen. Streptozocin biosynthesis may involve nitrous acid, another potent mutagen (2). It has been said that bioactive molecules, produced by bacteria and fungi, are no longer sought only for their potential use but also as a spur to the organic chemist to show what range of molecular structures are possible (3).

*3. Extracellular digestion allows bacteria to dissolve macromolecules.* An important process in many heterotrophs is the extracellular secretion of enzymes which digest insoluble materials, to give products that can be absorbed back into the

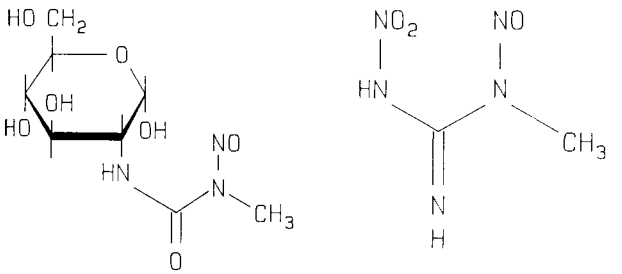


**Figure 1.** Chemical structures of streptozocin and *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine (NTG).

cell. With Gram-positive bacteria, the secretion process appears relatively simple: a proenzyme is targeted to the cell membrane and released by proteolytic cleavage of an $NH_2$-terminal signal sequence (4). In the case of Gram-negative cells, the process is complicated by the presence of an outer membrane. Therefore *Escherichia coli* is generally unable to export enzymes beyond the periplasm. However, many Gram-negative plant pathogens secrete pectinases, cellulases and proteases, presumably to macerate plant tissues. The application of molecular genetics to genera such as *Erwinia* (5) and *Xanthomonas* (Chapter 17) promises to reveal the special mechanisms that are responsible.

## B. Life in Extreme Environments

*1. Life on or in other organisms.* Many bacteria are adapted to use other organisms as an ecological niche: so exquisitely in some cases that they have become obligate pathogens and have proved refractory to man's attempts to culture them outside the cell or tissue. Perhaps the most famous example is the leprosy bacillus. Even the secrets of this refractory organism may soon be accessible through the achievements of recombinant DNA technology. *Mycobacterium leprae* genes have been cloned in *E. coli* (6) and recently there is a strong suggestion that many more will be well-expressed in the more closely-related streptomycetes (7). Intracellular parasitism, as in leprosy, provides pathogens with one form of defence against the host's immune system. Other organisms have evolved ways to change their surface antigens. Well-studied examples include phase variation in *Salmonella* and antigenic variation in *Neisseria* (Chapter 13).

A milder kind of host colonization is the induction of crown-gall tumours and "hairy roots" on dicotyledonous plants by *Agrobacterium tumefaciens* and *A. rhizogenes*. These bacteria have evolved a mechanism of genetic colonization which results in the insertion of DNA encoding production of complex amino acids (opines) into the plant. The opines can then act as sole carbon and nitrogen source for the *Agrobacterium* which caused the colonization (Chapter 18).

As well as having pathogenic life styles, bacteria are capable of many symbiotic relationships with eukaryotes. An intensively-studied example is the legume/*Rhizobium* interaction (Chapter 19), but other examples, which are receiving increasing attention, include cyanobacteria in lichens and sulphur oxidizers in gutless worms (8). The origin of chloroplasts and mitochondria from endosymbiotic bacteria has been proposed and extensively discussed by Margulis (9).

*2. Life in physicochemically extreme environments.* The archaebacterial kingdom has a penchant for extreme environmental niches. Halobacteria can grow in

2.5–4.25 M saline. Other archaebacteria such as the sulphur oxidizer, *Sulfolobus* and the extreme thermophile, *Thermoplasma* live in an extremely acidic environment (pH 1 and lower). This habitat is also shared by the eubacterial sulphur oxidizers, e.g. *Thiobacillus* spp. Another, though less tolerant, group of acidophilic eubacteria are the lactic acid bacteria. Both the sulphur oxidizers and the lactic acid bacteria generate the extreme environments within which they live by the production of acids (sulphuric and lactic respectively) as a waste product. This strategy has given the lactic acid bacteria the leeway to lose many of their biosynthetic capabilities because, presumably, they can be sure of finding the necessary nucleic acid bases, amino acids and growth factors in the environment that they have "poisoned" for their competitors. A number of bacteria (e.g. *Alcaligenes*) can also grow in extremely alkaline environments. How they manage to maintain a proton gradient across their cell membrane is still an open question.

Extreme thermophiles are found in both the eubacteria (*Thermus*) and the archaebacteria (*Thermoplasma*, *Thermococcus*, etc.), where growth at 80°C is commonplace and up to 105°C possible. Some years ago, there was a report of bacteria living at 250°C in deep sea black smokers. However, this observation is disputed from both technical and theoretical points of view (10).

As a final example of a bacterium able to survive in an extreme environment, I include *Deinococcus*. Organisms of this genus, and of a related genus *Deinobacter*, can tolerate extremes of ionizing radiation. However, perhaps surprisingly, they are susceptible to some chemical mutagens. This has rendered them amenable to genetic analysis, and a transformation system has been developed in *Deinococcus radiodurans*. Analysis of the mechanisms of DNA repair has shown that a number are operating concomitantly. Also there appear to be four copies of the chromosome in each cell (11).

## III. TOWARDS A PHYLOGENY OF BACTERIA

### A. Construction of the Tree

The development of 16S and 5S rRNA oligonucleotide cataloguing and, more recently, of rapid RNA sequencing has allowed progress towards an objective and consistent bacterial phylogeny, well reviewed by Woese (12) and Stackebrandt (13). Ribosomal RNA is a particularly good "molecular clock" because it performs the same function in all organisms and is essential under all physiological conditions. Therefore, sequences have altered with time at a slow enough pace to allow the comparison of genera which have little overall DNA homology.

Figure 2 is a diagram of the proposed universal phylogenetic tree of bacteria, and should be studied in conjunction with Table I. It is obvious at first glance

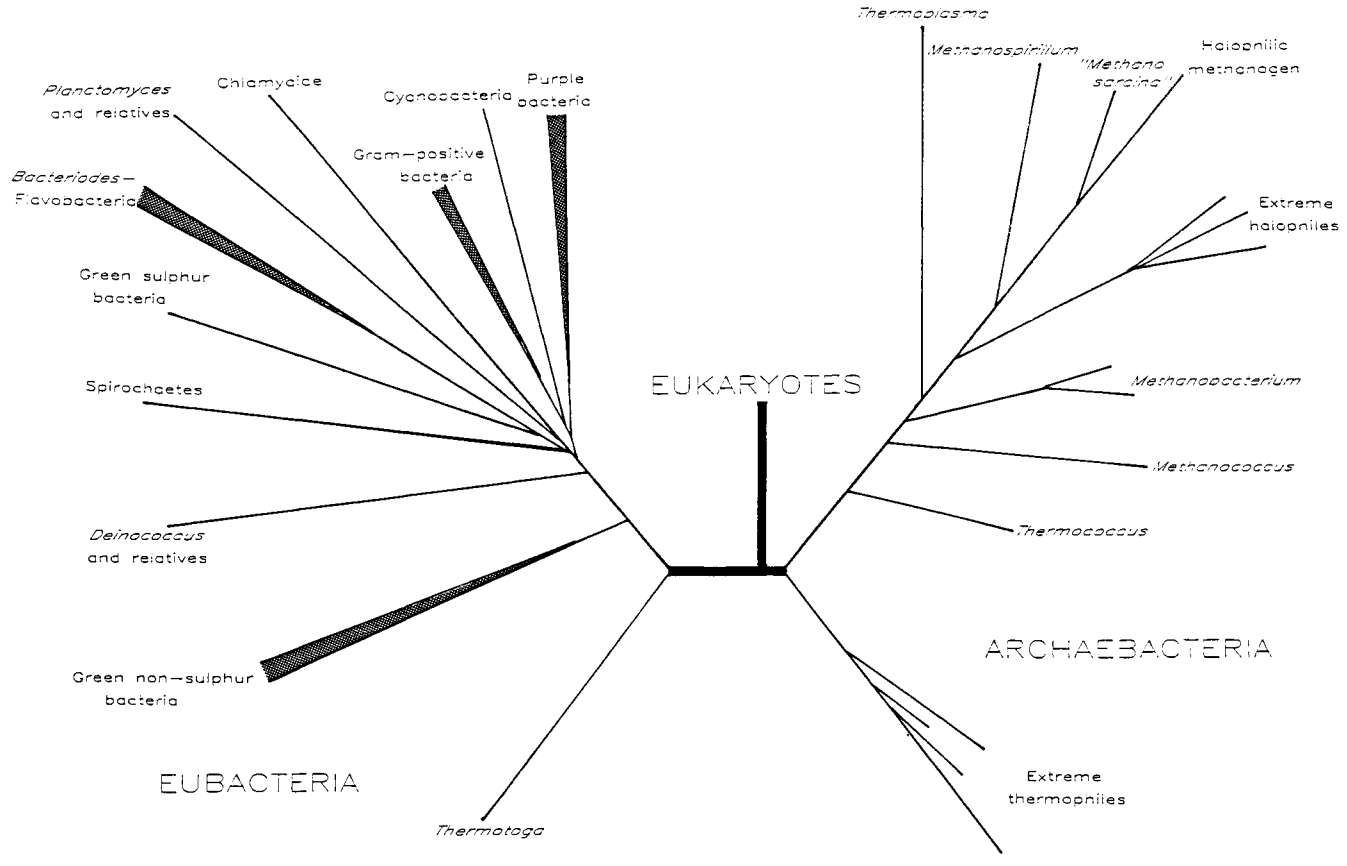
Thermoplasma
Methanospirillum
"Methano sarcina"
Halophilic methanogen
Extreme halophiles
Methanobacterium
Methanococcus
Thermococcus
ARCHAEBACTERIA
Extreme thermophiles
Thermotoga
EUBACTERIA
Green non-sulphur bacteria
Deinococcus and relatives
Spirochaetes
Green sulphur bacteria
Bacteriodes-Flavobacteria
Planctomyces and relatives
Chlamydiae
Cyanobacteria
Purple bacteria
Gram-positive bacteria
EUKARYOTES

**Figure 2.**

**Table I**

The eubacteria and archaebacteria phyla and their major subdivisions. Taken from Tables 2 and 14 of Woese (12) by permission of the author with additions from ref. 29

Eubacteria and their subdivisions

Purple bacteria
- $\alpha$ subdivision
  Purple non-sulphur bacteria (*Rhodobacter, Rhodopseudomonas*) rhizobacteria, agrobacteria, rickettsiae, *Nitrobacter, Thiobacillus* (some), *Azospirillum, Caulobacter*
- $\beta$ subdivision
  *Rhodocyclus* (some), *Thiobacillus* (some), *Alcaligenes, Bordetella, Spirillum, Nitrosovibrio, Neisseria*
- $\gamma$ subdivision
  Enterics (*Acinetobacter, Erwinia, Escherichia, Klebsiella, Salmonella, Serratia, Shigella, Yersinia*), vibrios, fluorescent pseudomonads, purple sulphur bacteria, *Legionella* (some), *Azotobacter, Beggiatoa, Thiobacillus* (some), *Photobacterium, Xanthomonas*
- $\delta$ subdivision
  Sulphur and sulphate reducers (*Desulphovibrio*), myxobacteria, bdellovibrios

Gram-positive eubacteria
- A. High (G + C) species
  *Actinomyces, Streptomyces, Actinoplanes, Arthrobacter, Micrococcus, Bifidobacterium, Frankia, Mycobacterium, Corynebacterium*
- B. Low (G + C) species
  *Clostridium, Bacillus, Staphylococcus, Streptococcus*, mycoplasmas, lactic acid bacteria
- C. Photosynthetic species
  *Heliobacterium*
- D. Species with Gram-negative walls
  *Megasphaera, Sporomusa*

Cyanobacteria and chloroplasts
*Oscillatoria, Nostoc, Synechococcus, Prochloron, Anabaena, Anacystis, Calothrix*

Spirochaetes and relatives
- A. Spirochaetes
  *Spirochaeta, Treponema, Borrelia*
- B. Leptospiras
  *Leptospira, Leptonema*

Green sulphur bacteria
*Chlorobium, Chloroherpeton*

Bacteroides, flavobacteria and relatives
- A. Bacteroides group
  *Bacteroides, Fusobacterium*
- B. Flavobacterium group
  *Flavobacterium, Cytophaga, Saprospira, Flexibacter*

*continued*

**Figure 2.** Universal phylogenetic tree of the bacteria. Line lengths are proportional to evolutionary distances except that the thick black lines represent five times the distance of the thin lines. The figure is compiled from Figs. 4, 11 and 13 of Woese (12) with permission of the author and this reference should be consulted for details of the method of construction of the trees. This figure should be consulted in conjunction with Table I.

**Table I**
*Continued*

Planctomyces and relatives
- A. Planctomyces group
  *Planctomyces, Pasteuria*
- B. Thermophiles
  *Isocystis pallida*

Chlamydiae
*Chlamydia psittaci, C. trachomatis*

Radio-resistant micrococci and relatives
- A. Deinococcus group
  *Deinococcus radiodurans*
- B. Thermophiles
  *Thermus aquaticus*

Green nonsulphur bacteria and relatives
- A. Chloroflexus group
  *Chloroflexus, Herpetosiphon*
- B. Thermomicrobium group
  *Thermomicrobium roseum*

Archaebacterial subdivisions

Extreme halophiles
*Halobacterium, Halococcus morrhuae*

Methanobacter group
*Methanobacterium, Methanobrevibacter, Methanosphaera stadtmaniae, Methanothermus fervidus*

Methanococcus group
*Methanococcus*

"Methanosarcina" group
*Methanosarcina barkeri, Methanococcoides methylutens, Methanothrix soehngenii*

Methanospirillum group
*Methanospirillum hungatei, Methanomicrobium, Methanogenium, Methanoplanus limicola*

Thermoplasma group
*Thermoplasma acidophilum*

Thermococcus group
*Thermococcus celer*

Extreme thermophiles
*Sulfolobus, Thermoproteus tenax, Desulfurococcus mobilis, Pyrodictium occultum*

that the bacteria which have received most attention, and indeed to which most of this book is dedicated, namely the Gram-positive and the purple bacteria, form only a narrow part of the phylogenetic tree. It should, however, be noted that some branches of the tree contain very few members.

## B. Genetic Exchange Between the Branches of the Tree

It turns out that the bacteria used to study exchange of genetic material between genera (Chapter 2), in the form of plasmids or transposons, are in fact relatively closely related. The ability of the Inc P group plasmids to transfer to a large number of bacteria of very different properties and appearance suggests that all Gram-negative bacteria are tightly affiliated. However, the bacteria in question all fall into the purple $\alpha$, $\beta$ and $\gamma$ groups. When RP4 was transferred to a $\delta$ group bacterium (*Myxococcus xanthus*), it could only be maintained if it was inserted into the chromosome (14). The recently identified plasmids that appear to be able to replicate autonomously in both purple Gram-negative and Gram-positive organisms (15), and transposons that can transpose in both bacterial groups (16), may be better examples of promiscuous genetic elements. Plasmids have been identified in bacteria from many other branches of the eubacterial and archaebacterial kingdoms, but their genetic mobility is still essentially unstudied.

The construction of a phylogenetic tree gives rise to some interesting taxonomic problems (Chapter 20). For instance: What is a genus and what is a species in the bacterial world? If we ignore plasmids, phages and transposons, which have evolved to cross taxonomic groups as mentioned above, the ability to exchange and integrate chromosomal DNA via homologous recombination might still be used as a definition of species (albeit one with severe experimental restrictions). Curiously, this ability differs in different branches of the tree. Chromosomal DNA can be exchanged and assimilated by recombination, given enough selective pressure, between what are classed as different genera in the enterics, e.g. *Salmonella* and *Escherichia*. In contrast, within the genus *Streptomyces*, where species separation is very shallow using numerical taxonomic assignment, some species clusters contain strains that show little DNA homology with each other (17). This paradox is yet to be resolved.

## C. The Archaebacteria

The identification of the archaebacteria as a separate kingdom in 1977 (12) stimulated a great deal of interest in these otherwise quite disparate bacteria.

Their possession of many unusual features—lipids not previously seen in the bacterial world, and unusual cofactors like cofactor M—was not perhaps surprising considering their unusual life styles (see above). However, the discovery of singular features of the transcriptional/translational machinery of these organisms emphasized their separation from the eubacterial kingdom. A particular singularity was the discovery of introns in *Sulfolobus solfataricus* and *Halobacterium volcanii* (18). However, the bizarre nature of this discovery was mitigated by the discovery of an intron in the coliphage T4 (19). Attempts to develop genetic analysis of both the *Halobacteriaceae* and the *Methanobacteriaceae* have begun. Recent papers have reported bacteriophage transfection and plasmid transformation of *Halobacterium* spp. and low level transformation of chromosomal markers in *Methanococcus voltae*. In addition, Mevarech and Werczberger have reported a natural system of genetic exchange in *H. volcanii* (20).

## IV. REINVENTION THROUGHOUT THE PHYLOGENETIC TREE

One problem with developing a phylogeny of bacteria is to take account of the possibility that DNA has been exchanged between bacteria from different genera and different branches of the tree. As discussed above, plasmids and transposons can potentially be exchanged between different branches. Drug resistance genes may have been exchanged between the producers of antibiotics, mostly Gram-positive bacteria, and the purple group of bacteria (though the number of examples on which this rests has recently been shown to be smaller than previously thought; 21). The following discussion is intended to highlight areas where either reinvention or gene transfer may have taken place, based on our present knowledge of bacterial phylogeny.

### A. Endospores Have Probably Been Invented Only Once, but Exospores Several Times

Many bacteria produce resting structures and/or specialized dispersal stages. It has now become clear that endospores (Chapter 11) are produced by a phylogenetically closely knit group, namely the low (G + C) Gram-positive bacteria that includes *Thermoactinomyces*, a genus once classified with the Actinomycetes.

Exospore formation or cyst formation—the terminology is confused in different systems—appears to have been reinvented many times. The cyanobacteria, purple bacteria and Gram-positives all contain a variety of examples. The *Myxobacteriales* (purple bacteria) and the *Streptomycetaceae*