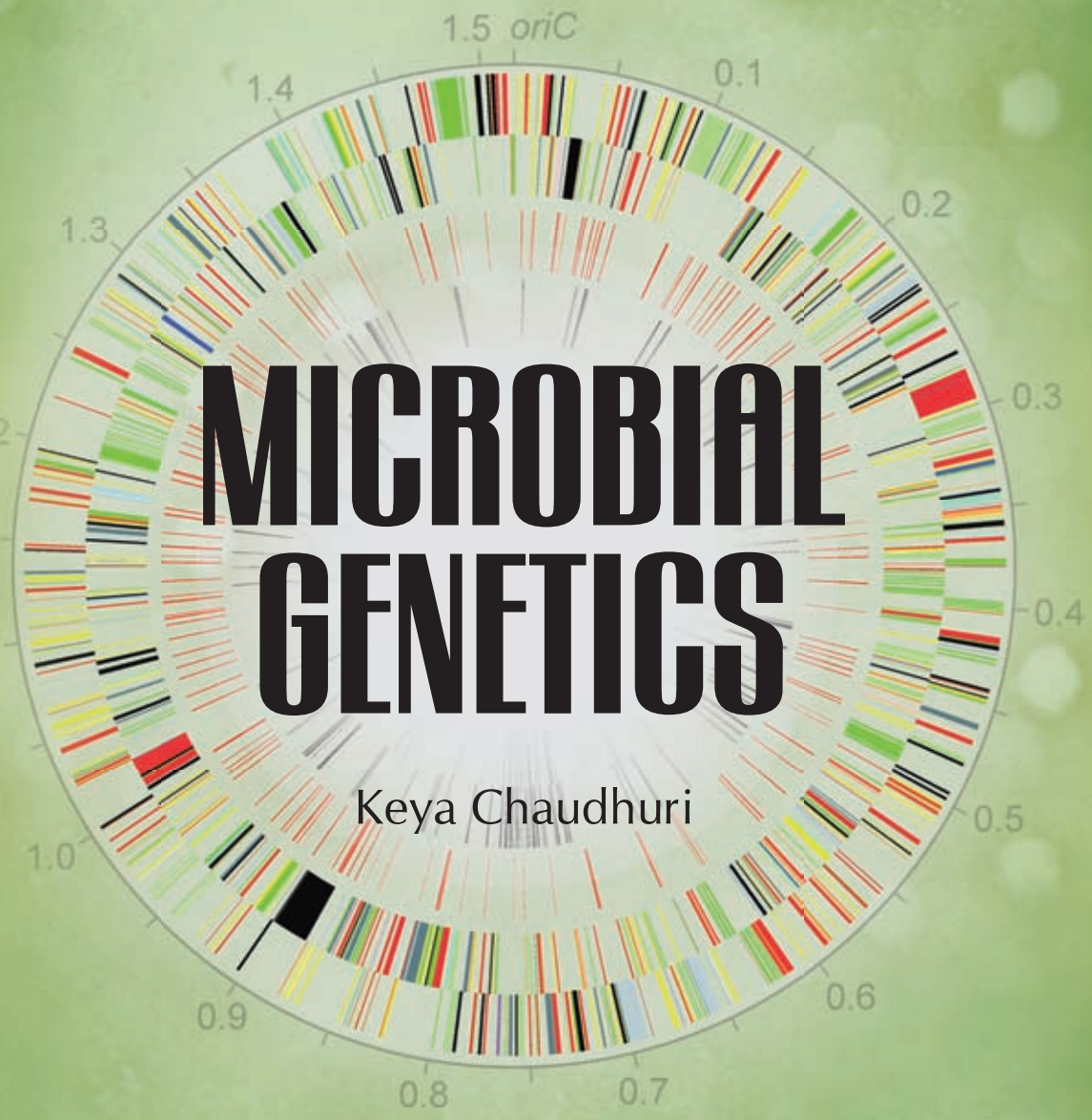

MICROBIAL GENETICS

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Preface

The science of genetics began with the work of Gregor Mendel, an Austrian monk, as early as the middle of the 19th century. Mendel's experiments with selective cross-breeding of pea plants established the fundamental principles of heredity. He described certain laws of inheritance and showed that certain physical characteristics called traits, such as flower colour, seed colour, seed shape, stem length, and so on, are passed on from generation to generation; some traits called recessive are masked by dominant traits and most traits are inherited independently of others. For example, pea flowers are either purple or white, and intermediate colours do not show up upon cross-breeding. However, Mendel's findings remained unnoticed till the 20th century when geneticists experimenting on fruit flies recognized his work; it was shown that there are genes in chromosomes. Specific genetic alterations could also be linked to the change of physical characteristics of the organism, that is, genotype–phenotype relations could be established. However, the bacterial genetics studies were the first to bring in the chemical nature of the gene as well as the mechanisms by which the genotype determines the phenotype. The journey began with the breakthrough in the 1940s which showed that bacteria can be a genetic tool, and since then many of the principles of genetics as well as recombinant DNA technology have been developed around bacteria. *Escherichia coli* was the primary focus in all these studies, and some of the genetically useful bacterial viruses called bacteriophages were also involved in elucidating genetic principles. It may be recalled that the first genome to be sequenced completely was a bacterial genome. Although the sequencing of *E. coli* genome was started before, the credit of the first genome to be sequenced goes to *Haemophilus in uenzae*. Understanding the principles of bacterial and bacteriophage genetics is therefore extremely important and needs to be emphasized for understanding the areas of molecular biology and recombinant DNA technology.

Importance of Bacteria and Bacteriophages

Biologists use simpler model systems which are easy to grow in the laboratory and can easily be manipulated. This is because it is difficult to conduct experiments in complex organisms like primates and it is unethical to conduct some of the experiments on human. Bacteria are relatively simple organisms. They can be both grown easily in the laboratory and also manipulated easily. They serve as a model system to understand the cellular functions and processes in more complex organisms.

Bacteria are important not only as a laboratory tool, but also as an important constituent of life on earth. They play an essential role in ecology of the earth, and have a central role in nitrogen fixation and carbon cycle. They are used for degrading certain chemicals and toxic products, cleaning up waste, and leaching of metals from their ores, and, thus, are industrially important. As many bacteria can thrive in extreme environments, they are the sources of thermostable enzymes. Some bacteria like symbiotic bacteria are found in humans and other organisms, and are beneficial for them. However, a number of bacteria exist which are pathogenic to human. It is necessary to identify and study these pathogenic organisms so that drugs can be developed and human beings can be protected against diseases. Bacteria, phages, and the viruses that infect bacteria are worth studying as they serve as the source of many useful enzymes.

As have started understanding these organisms, one of the developments in these areas has been the availability of complete genome sequences of many bacteria and phages. However, a substantial part of the protein coding regions are of unknown function. Also thousands of different bacteria are known, many of which are even unculturable. Studies on bacteria will continue which will enable us to understand, control, and benefit from this life form, and bacterial molecular genetics will be an essential tool in this endeavour.

The Three Kingdoms of Life: Eukaryotes, Bacteria, and Archaea

When life was first classified by scientists, it included only two divisions: animals and plants. Gradually with the discovery of other life forms, scientists began to recognize five kingdoms of life: animals, plants, fungi, protists, and bacteria. These five kingdoms were generally grouped into two: the eukarya, those having nucleus in their cells and prokarya, those devoid of nucleus in their cells. In eukarya, animals, plants, protists, and fungi were included because they had nucleus inside the cell and DNA was enclosed inside the nucleus with a nuclear membrane around it. Bacteria were included in prokarya as their DNA is present in the cytoplasm surrounded by some basic proteins which is supposed to form a nucleoid-like structure.

Later on, new insights into molecular biology changed this view of life. Another type of organisms which were earlier included in bacteria were found to have their 16S ribosomal RNA sequences differing widely from bacterial 16S ribosomal RNA sequences. The 16SrRNA sequences of archaea were much near to the rRNA sequences found in

eukaryotes. They had certain properties that differed from bacteria and eukarya, and had to be classified into a separate class now known as archaea. The brief characteristics of these three kingdoms of life is given in Figure 1 and described in the ensuing paragraphs.

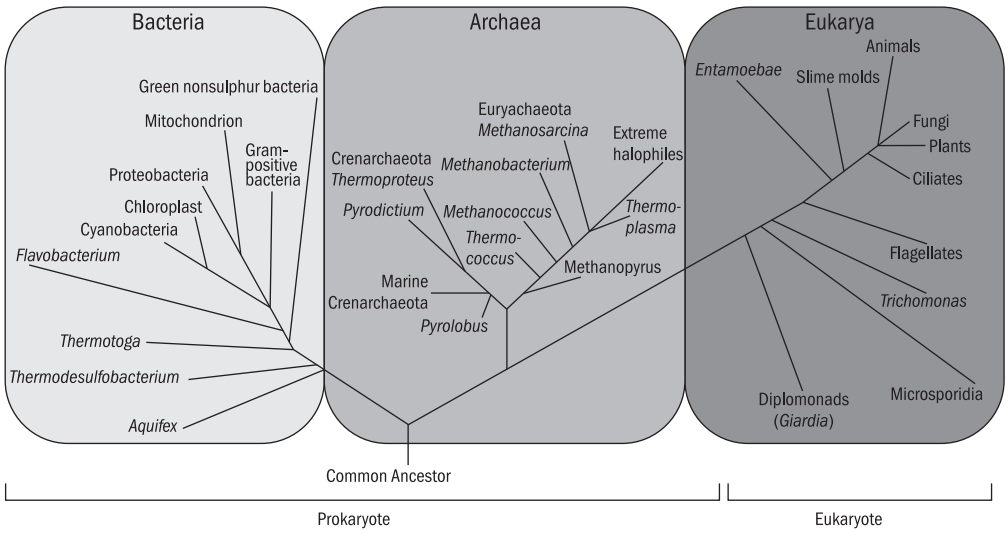


Fig. 1 *Three domains of life*

Archaea

- Archaea are microscopic unicellular organisms.
- They neither have membrane-bound structures or organelle nor do they have a nucleus. Structurally, they resemble bacteria and are included under prokaryotes.
- Biochemically archaea differ from bacteria as also from eukaryotes. So they are placed in a kingdom of their own.
- Archaea can exist in a variety of shapes such as rods, spheres, triangles, discs, plates, and cup-shapes.
- The ribosomal RNA sequences of archaea are more closely related to eukarya.
- The archaea, unlike bacteria, have histone proteins associated with their DNA as eukaryotes.
- Archaea can live in extreme environments such as thermal vents, hypersaline water, sulphur springs, high pressure regions of ocean, and also in ordinary temperatures and salinities. They can live under the conditions where other types of organisms cannot survive. They are often called extremophiles.
- Archaea do not require oxygen as do animals and do not perform photosynthesis like plants; instead many archaea are methanogenic, that is, they give off methane gas as a byproduct of their activities.

Bacteria

- Bacteria lack membrane-bound nucleus. The DNA is present as a circular molecule with no associated histones, condensed to a region in cytoplasm, and is called nucleoid.
- Bacteria are very small in size and cannot be studied as isolated cell. Instead, they are studied on nutrient agar plates where they form colonies. A bacterial colony contains millions of cells, all having descended from a single ancestor.
- Bacteria differ in shapes. They may be round shaped (cocci), rods (bacilli), helical, or spiral shaped (spirochetes).
- Bacteria differ greatly in their physical appearance as colonies or under microscope.
- Cyanobacteria, formerly called blue green algae, are classified under bacteria but they have chlorophyll and are of filamentous form. The antibiotic producing streptomyces species form hyphae and stocks of spores like fungi. Another genus called Myxococcus can exist as a free-living organism and can also aggregate to form bodies like slime molds.
- No organelles or membrane-bound structures are found in bacteria.
- A large number of ribosomes are present which are complexes of RNA and protein, and are required for protein synthesis.

Table 1 describes briefly the prokaryotic structures and their functions.

Table 1 Prokaryotic structures and functions

Structure	*Description*	*Function*
Plasma membrane	Basic structure that surrounds bacterial cell; made of phospholipid bilayer	Serves as a permeability barrier; selectively transports nutrients and waste products; site for many metabolic processes
Nucleoid	Irregularly shaped nucleoplasmic region inside the cell cytoplasm; it is not bounded by any membrane	Localization of prokaryotic chromosome
Ribosomes	Bead like structures; made of RNA and protein	Protein synthesis
Inclusion bodies	Aggregates of stainable substances	Storage of materials (example glucose, phosphates) in polymer form
Periplasmic space	The region between plasma membrane and outer membrane in Gram-negative bacteria	Storehouse of hydrolytic enzymes and proteins required for transport
Cell wall	Contains peptidoglycan which is a complex of polysaccharide polymers cross-linked via short amino acid chain	Determines the shape of bacteria; protects from cellular lysis
Capsules and slime layers	Gelatinous polysaccharide sheath or layer surrounding the cell wall	Attachment to surfaces, resistance to phagocytosis
Pili	Hair-like structure composed of proteins; present on bacterial cell surface	Required for bacterial conjugation; attachment to surfaces, example colonization to host surface

Table 1 *Continued*

Structure	*Description*	*Function*
Fimbriae	Hair-like structure on the cell surface	Attachment to surfaces
Flagella	Long thin appendage protruding out of the cell	Propel bacteria; responsible for movement
Endospore	A dormant, tough, and non-reproducing structure	Survival under environmental stress

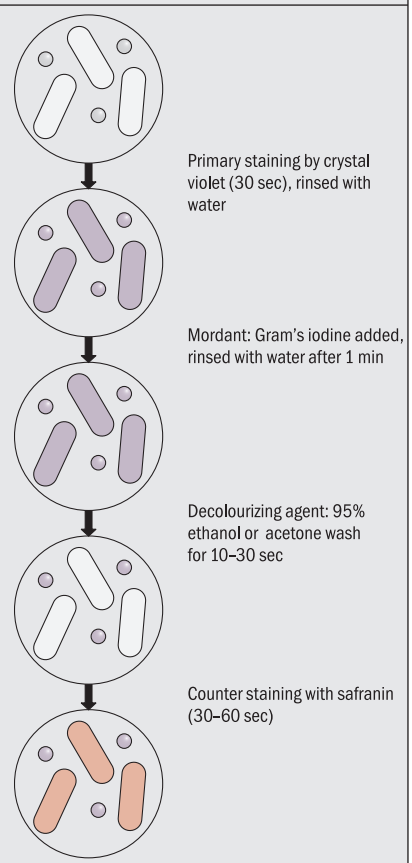
Gram-positive and Gram-negative Bacteria

Bacteria can be further subdivided into two classes, viz. Gram-positive and Gram-negative. This classification is based on the reaction of bacteria towards Gram-stain. Box 1 explains the Gram-staining procedure. After staining, the Gram-negatives appear as pink while Gram-positives appear as deep blue.

Box 1 *The Gram-staining procedure*

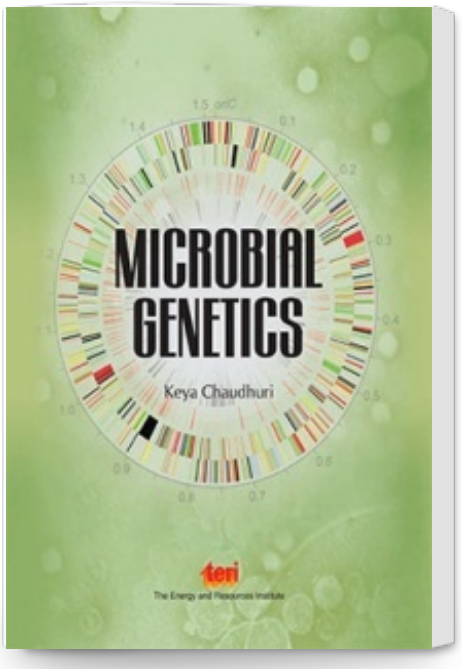
The Gram stain was developed by Christian Gram in 1884 and is still in use today. According to the Gram-staining procedure, the smear of bacteria is at first treated with the basic dye crystal violet followed by treatment with iodine solution. The iodine solution increases the interaction between the cell and the dye. The smear is next washed with acetone or alcohol. In the case of Gram-negatives, the crystal violet is washed out and they appear colourless while the Gram-positive bacteria retain the crystal violet stain. Finally, the smear is counter-stained by another dye Safranin which colours Gram-negatives pink to red and Gram-positives retain the purple blue colour.

The difference between the reactions of Gram-positive and Gram-negative bacteria towards crystal violet staining is due to the differences in the structure of the cell wall of these two types of organisms. The peptidoglycan structure, which is very thick and highly cross-linked in Gram-positives, is involved in the retention of crystal violet. Following crystal violet and iodine treatment when Gram-positive bacteria are treated with alcohol or acetone, the latter is thought to shrink the pores of the peptidoglycan and help in dye retention. On the other hand, the peptidoglycan in Gram-negatives is a thin layer, and less cross-linked and has larger pores. The alcohol treatment further may extract lipids from the cell wall making the porosity to increase more. Hence, the dye is easily removed from Gram-negative bacteria.



Procedure for Gram-staining

Microbial Genetics



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